Journal of Vascular Research

Abstracts

25th Conference of the European Society for Microcirculation

Integrating Vascular Biology and Medicine: Basic and Clinical Science

(www.karger.com/jvr_45_S2_08)

Budapest, Hungary, August 26–29, 2008

Guest Editor
Akos Koller, Pécs/Budapest/Valhalla, N.Y.

379 abstracts, PDF (1400 Kb, 2008)

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P.O. Box, CH–4009 Basel (Switzerland)
eISBN 978–3–8055–8636–8

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POSTER PRESENTATION ABSTRACTS

Tuesday, August 26th

PAG	Angiogenesis
PVR	Vascular remodelling
PAS	Atherosclerosis
PCA	Clinical assessment of microvascular function
PPC	Pulmonary microcirculation
POT	Oxygen transport to tissue
PRB	Haemorheology and red blood cells
PSH	Shock
PLY	Lymphatics

Wednesday, August 27th

PDO	Diabetes mellitus, obesity
PHT	Hypertension
PEC	Endothelial cell biology and function
PPE	Permeability and glycocalyx/endothelial surface layer
PIN	Inflammation and leukocyte function
PHP	Haemostasis and platelet function

PMI	Microvascular imaging

Thursday, August 28th

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PTC	Vascular tone control
PCD	Cardiac dysfunction
PRM	Renal microcirculation
PCM	Cerebral microcirculation
PVA	Varia

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AWARD PRESENTATION ABSTRACTS
Wednesday, August 27th
Awards Presentation

MALPIGHI AWARD LECTURE

THE ROLE OF MECHANICAL FACTORS IN ANGIOGENESIS IN SKELETAL MUSCLE.

Olga Hudlicka
Univ of Birmingham, UK

EXERCISE-INDUCED ANGIOGENESIS IN SKELETAL MUSCLE

O Hudlicka [1], MD Brown [2], M Milkiewicz [1,2]
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Exercise training results in capillary growth in muscles predominantly involved in the type of training: in those composed mainly of oxidative fibres with endurance training, in those composed of glycolytic fibres in sprint or exhaustive exercise. Using chronic electrical stimulation of one muscle group as a model of exhaustive exercise and comparing its effect with long term vasodilator (prazosin) induced increases in blood flow, we demonstrate the importance of d blood flow and increases capillary shear stress in the initiation of capillary growth. High blood flow in muscles of stimulated and prazosin treated animals results in increased capillary proliferation and raised expression of VEGF in capillaries. Increased shear stress found in 2 days stimulated muscles even at rest was linked with raised expression of VEGF and VEGFR2 protein. Inhibition of eNOS by long-term administration of L-NNA suppressed stimulation-induced increases in capillary shear stress and VEGF and VEGFR2 expression and eliminated capillary proliferation and increase in capillary/fibre ratio. It also suppressed capillary growth in muscles of prazosin treated mice (Williams et al. J. Physiol. 470:445-454). Thus nitric oxide plays an important role in increased blood flow/shear stress induced angiogenesis.
Supported by British Heart Foundation

LARS-ERIK GELIN CONFERENCE TRAVEL AWARDS (AWLE)

AWLE.1

PERMEABILITY TO WATER IS INCREASED IN GLOMERULAR AND SYSTEMIC MICROVESSELS OF MALE MUNICH WISTAR FROMTER RATS.

J Ferguson, CR Neal, DO Bates, SJ Harper, AH Salmon
Microvascular Research Laboratories, Bristol Heart Inst., Dept. of Physiology &Pharmacology, Univ. of Bristol, UK.

Cardiovascular disease is the leading cause of death in the western world. Recently albuminuria has been shown to predict cardiovascular mortality and has been proposed to reflect systemic endothelial dysfunction. (Yuyun Int J Epidemiol 2004; 33:189-198). The Munich Wistar Fromter (MWF) rat strain develops spontaneous albuminuria and therefore is an interesting experimental model to study alterations of endothelial function under conditions of increased albuminuria. Urine from male MWF rats (15-24 wks) was collected using metabolic cages and the protein:creatinine ratio measured. (MWF:15.5±1.67 n=14 Wistar:1.65±0.22 n=6, $p<0.0001$). Hydraulic conductivity (L_p) was measured in individually perfused microvessels of the mesentery. Male MWF rats had significantly increased L_p (cm $s^{-1}cmH_20^{-1}$) compared with age matched Wistar controls. (MWF: $6.39\pm1.75\times10^{-7}$ n=7; Wistar: $2.27\pm0.44\times10^{-7}$ n=8, p=0.02). Glomerular ultrafiltration co-efficient (L_pA/V_i) was measured using an oncometric assay. (Salmon J Physiol 2006; 570(1):141-56). The increase in systemic L_p was accompanied with an increase in glomerular L_pA/V_i. (MWF: 1.27±0.12 $min^{-1}mmHg^{-1}$ n=9; Wistar: 0.81±0.14 $min^{-1}mmHg^{-1}$ n=3, p=0.065). These results suggest the link between glomerular albuminuria and systemic cardiovascular disease is accompanied by similar defects in the barrier function of the microvessel wall.
Supported by BHF.

AWLE.2

LOW-DOSE METFORMIN THERAPY ATTENUATES LEFT VENTRICULAR DYSFUNCTION AND REDUCES MORTALITY IN A MURINE MODEL OF HEART FAILURE

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Clinical studies indicate that metformin protects against cardiovascular disease in patients with diabetes and metabolic syndrome by actions that cannot be solely attributed to its glucose-lowering effects. We sought to investigate the potential cardioprotective effects of an acute, low-dose metformin treatment (i.e. 286-fold less than the maximum anti-hyperglycemic dose) in a murine model of heart failure (HF). Mice were subjected to myocardial ischemia for 60 min followed by reperfusion. Metformin (125 µg/kg) or vehicle (saline) was administered as a single bolus (i.v.) at the time of reperfusion and then daily (intraperitoneal) for 4 wks. Administration of metformin reduced infarct size by 30% ($p<0.001$) at 24 hrs of reperfusion and significantly ($p<0.01$) improved LV function at 4 wks of reperfusion without altering blood glucose levels. At 4 wks of reperfusion, metformin therapy significantly ($p<0.05$) increased the phosphorylation status of AMPK and eNOS, increased the expression of PGC-1 , and improved mitochondrial ATP synthesis and oxygen consumption in the hearts of treated mice. In an additional group of mice subjected to a complete occlusion of the left coronary artery, metformin reduced mortality by 50% ($p<0.05$). These findings suggest that low-dose metformin therapy may have a practical clinical use for the treatment of HF. This work was supported by the National Institutes of Health (HL-060849-08 to D.J.L. and F32 DK 077380-01 to J.W.C.) and the American Diabetes Association (7-04-RA-59 to D.J.L.).

AWLE.3

NEUROTROPHIN-3 (NT-3) PROMOTES REPARATIVE NEOVASCULARIZATION AND BLOOD FLOW RECOVERY IN A MOUSE MODEL OF LIMB ISCHEMIA

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Neurotrophins regulate neuronal survival and regeneration. Non-neural functions of neurotrophins include angiogenesis promotion by NGF and BDNF and NT-3 involvement in heart development. The possibility that NT-3 promotes angiogenesis has not been evaluated yet. Therefore, we investigated the pro-angiogenic potential of NT-3 by adenovirus(*Ad*)-mediated-*NT-3* over-expression in a mouse model of limb ischemia. We preliminary found that capillaries and arterioles endothelial cells(EC) express trkC in skeletal muscles, thus suggesting their responsiveness to NT-3.

Limb ischemia was induced by left femoral artery occlusion on anaesthetized CD1 mice and *Ad-NT-3*(10^8p.f.u.) was injected in ischemic adductors. At day3 post-ischemia the underpinning molecular pathways were analysed on muscles. Blood flow(BF) recovery to the ischemic foot was measured by Colour Laser Doppler on day7 and 14. Histology and IHC analyses of muscles were performed after 3(EC proliferation) and 14days (capillary and arteriole densities) post-ischemia.

Ad.NT-3 induced phosphorylation of trkC receptor, Akt and eNOS($P<0.05$ for comparisons vs. control), while it did not change VEGF-A expression. *Ad.NT-3* increased the number of proliferating EC($P<0.05$) and the density of both capillaries($P<0.01$) and arterioles($P<0.05$) in ischemic adductors and it improved BF recovery($P<0.05$ vs. control on both day7 and 14). NT-3 could be considered as a candidate factor for treatment of ischemic vascular disease.

VAN LEEUWENHOEK DISTINCTIVE TRAVEL AWARD 2008

Jürgen Schymeinsky
Institute of Physiology, LMU Munich, Germany

INTERNATIONAL INSTITUTE FOR MICROCIRCULATION AWARD FOR THE BEST COMMUNICATION OF HOST COUNTRY

X
X
X

SERVIER AWARD

X

JVR/ESM Prize 2008 Award

TWO-PHOTON MICROSCOPY OF VITAL MURINE ELASTIC AND MUSCULAR ARTERIES. COMBINED STRUCTURAL AND FUNCTIONAL IMAGING WITH SUBCELLULAR RESOLUTION.

Journal of Vascular Research 44: 87-98 (2007)

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Fluorescence lifetime imaging (FLIM) provides a complementary contrast mechanism to fluorescence intensity and ratio imaging in intact tissue. With FLIM the time-resolved decay in fluorescence intensity of (interacting) fluorophores can be quantified by means of time correlated single photon counting (TCSPC). Here we focus on fluorescence lifetime imaging in intact blood vessels. Requisites for imaging in intact tissue are good penetration depth and limited tissue damage. Therefore, in this pilot-study, we performed TCSPC-FLIM using two-photon laser scanning microscopy to determine, with sub-cellular resolution, the fluorescence lifetime of two fluorescent probes. First, we focused on the nucleic acid dye SYTO41 in the various compartments of cells in vitro and in situ in the wall of intact mouse carotid arteries. Second, it was assessed whether the interaction of the lectin WGA-FITC with the endothelial glycocalyx affects its fluorescence lifetime. Results showed comparable mono-exponential fluorescence lifetimes of SYTO41 in the nuclei of cells in vitro and in situ. The slightly shorter fluorescence lifetime observed in the cytoplasm allowed discrimination of the nuclei. SYTO41 displayed strong mitochondrial staining, as was verified by the mitochondrion-specific probe CMXRos. In addition, mitochondrial staining by SYTO41 was accompanied by a green shift in emission. In the mitochondrial region, SYTO41 showed a highly bi-exponential and relatively fast decay, with two distinct lifetime components. It is hypothesized that the fitted bi-exponential decay can either be contributed to (1) the mathematical approximation of the fluorescence intensity decay or (2) the presence of free and DNA-bound SYTO41 in the mitochondrial compartment, leading to two lifetime components. The fluorescence lifetime of WGA-FITC decreased by approximately 25% upon binding to the endothelial glycocalyx. From this study, we conclude that FLIM offers an additional contrast mechanism in imaging intact tissue and provides information on binding status between a probe and its ligand. Copyright 2007 Wiley-Liss, Inc.

PL1

ANGIOTENSIN II, NO AND ROS: AUTONOMIC AND CEREBROVASCULAR EFFECTS IN HEART FAILURE AND DIABETES

Irving H. Zucker
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Chronic heart failure (CHF) is characterized by activation of the sympathetic nervous system and the renin angiotensin II (Ang II) system. These compensatory mechanisms are beneficial in the early stages of CHF. However, prolonged activation exacerbates the CHF state causing further deterioration in cardiac and peripheral organ function. The mechanism by which central regulation of sympathetic outflow is controlled in the CHF state is not completely understood. Our laboratory has investigated the role of Ang II and reactive oxidant stress (ROS) in the regulation of sympathetic tone in CHF. We have found that in most areas of the mid and hind brain that regulate sympathetic tone, both angiotensin type 1 (AT1) and type 2 receptors (AT2) are up regulated. For AT1 receptors this up regulation is totally dependent on Ang II binding. Chronic administration of the AT1 receptor antagonist losartan completely prevents AT1 receptor up regulation in the rostral ventrolateral medulla (RVLM) and paraventricular nucleus (PVN) of rabbits with pacing-induced CHF. The molecular mechanism by which AT1 receptors are up regulated in heart failure or following increases in plasma Ang II involves the stimulation of the transcription factor Activator Protein 1 (AP1). AP1 is formed by the dimerization of two c-Jun or a c-Jun and a c-Fos molecule. In rabbits with CHF, enhanced AP1 binding to the promoter region of the AT1 receptor gene was observed along with an increase in c-Jun and Jun Kinase (JNK). The up regulation in AT1 receptor protein could be inhibited by JNK inhibitors or losartan but not by inhibitors of ERK or p38 MAPK. Ang II signaling involves an NAD(P)H Oxidase mediated superoxide production in the RVLM, nucleus of the tractus solitarius (NTS) and PVN. CHF rabbits exhibit an increase in several of the protein subunits of the NAD(P)H Oxidase enzyme. The sympatho-excitation in the CHF state can be reduced following central treatment with free radical scavengers or NAD(P)H Oxidase inhibitors. Ang II may also mediate an increase in sympathetic neuronal excitability by causing a chronic down regulation of potassium channel proteins. In the CHF state or in neuronal cell cultures incubated with Ang II, the Kv4.3 channel protein was observed to be downregulated along with a decrease in potassium channel current. Nitric Oxide (NO) may play an important role in the sympatho-excitation in CHF. Nitric Oxide Synthase (NOS) is downregulated in the PVN and RVLM in the CHF state. NO, being a sympatho-inhibitory substance may contribute to sympatho-excitation when its synthesis is decreased. Interventions that increase NO biosynthesis such as adenoviral gene transfer, exercise training and statin treatment all reduce the sympatho-excitation in CHF. There are important links and commonalities between neural regulation of sympathetic tone in heart failure and vascular reactivity of the cerebral microcirculation in diabetes. Recent studies from our group have shown that stimulation of NMDA receptors with glutamate activate an NO dependent mechanism that vasodilates nearby vessels. This mechanism is dramatically impaired in animals with Type 1 diabetes. In addition, the regulation of AT1 receptor signaling in cerebral vessels is similar to that in neurons.

Irving H. Zucker
Professor and Chair
Ph.D. 1972, New York Medical College
Major Interest: Cardiovascular Physiology
Specialty: Neural control of the circulation; angiotensin II; nitric oxide; reactive oxidant stress.
Our research focuses on the role of the central brain mechanisms on neurohormonal adjustments in cardiovascular regulation in the setting of experimental chronic heart failure. We are interested in the role of substances such as angiotensin II, nitric oxide and reactive oxidant stress as important mediators of sympathetic excitation in the setting of chronic heart failure. Molecular, cellular and whole animal techniques are used to understand abnormal cardiovascular and neural regulation in heart failure. In addition, we are extremely interested in the role of exercise training in ameliorating some of the abnormalities we have found in the central nervous system of animals with heart failure. These techniques are providing important new information about normal cardiovascular physiology and about the pathophysiology of heart failure and other cardiovascular diseases. Students in this laboratory learn surgical preparation of chronically instrumented animals and techniques for measuring a variety of cardiovascular parameters in awake, chronically instrumented animals. Molecular biological techniques such as Western blots, real time RT PCR and histological techniques are routinely used.

S1A
ROLE OF THE ARTERIOLES IN REGULATING THE CIRCULATION
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The purpose of this study is to re-examine the overall regulation of the circulation by introducing intrinsic regulatory mechanisms of the arterioles; specifically, autoregulation of blood flow, i.e. the tendency of blood flow to be independent of arterial pressure. We propose that at a very fundamental level, the interaction between intrinsic regulatory mechanisms of the heart (Starling's Law) and the autoregulatory mechanisms of the microcirculation determine arterial pressure and cardiac output. In the past, much emphasis has been placed on the role of the central nervous system and neural reflexes in regulation of the circulation via a "set point" for arterial pressure. We propose that neural control mechanisms modify, rather than set overall regulation, by altering factors such as heart rate and contractility, vascular resistance and venous compliance. A conceptual model based on this principle predicts the observed effects of decreasing vascular resistance, static exercise and dynamic exercise on cardiac output and arterial pressure. We suggest that adjusting supply to match demand by coupling intrinsic regulatory mechanisms of the heart to those of the arterioles would in principle more effectively maintain homeostasis of the tissues than if the central nervous system sets arterial pressure by adjusting supply and demand.

S1B
WILL LESSONS ABOUT MICROVASCULAR PERMEABILTY AND EXCHANGE HAVE TO BE RE-LEARNT?
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Between 1945 and 1980, advances in the analysis of biological transport processes, new experimental methods and many new observations led to more rigorous and more extensive investigations of the microcirculation and particularly microvascular exchange. Recent advances in endothelial cell biology have moved many of these developments into the shadows. More seriously, the increasing dependence on internet searches of the scientific literature that are restricted to the past 30-40 years means that much valuable early work is in danger of being forgotten. Serious implications of this are not limited to the advances in knowledge of microvascular permeability and exchange but also concern the interpretation of widely used methods of assaying changes in microvascular permeability and the interpretation of plasma and tissue clearance curves. An analysis will be presented of the most widely used method of estimating changes in vascular permeability (Miles assay). The limitations of the interpretation of plasma clearance curves will also be discussed. It is argued that careful mathematical modelling of integrative microvascular function can minimise erroneous interpretations.

S1C
THE ENDOTHELIAL GLYCOCALYX AS A BARRIER TO LEUKOCYTE-ENDOTHELIUM ADHESION AND TRANSVASCULAR EXCHANGE OF FLUID
HH Lipowsky, L Gao, R Sah and A Lescanic
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The endothelial surface layer has been shown to serve as a barrier to leukocyte (WBC) adhesion to the endothelium (EC). When the EC is activated by the chemoattractant fMLP, glycans are shed that appear to shield ligands for WBCs such as ICAM-1. The current study aimed to determine the extent to which glycans are shed in light of their contribution to the thickness of the layer and its ability to serve as a barrier to fluid exchange. To this end, shedding was delineated in post-capillary venules (rat mesentery) by measuring the intensity of the fluorescently labeled lectin BS-1, which binds preferentially to galactose, and infiltrates the glycocalyx. Superfusion with fMLP caused a 25% decrease in lectin binding within 30 min for arterioles, capillaries and venules. The apparent thickness of the layer was reduced by 20% during the shedding process, as evidenced by a decrease in thickness of the lectin stain from 0.9 to 0.7 microns. Changes in hydraulic conductivity of the layer were determined by analysis of the transit time of a bolus of fluorescent solutes (FITC) through the layer along the length of a venule, which revealed a 90% reduction. Thus, the structure of the EC glycocalyx may change dramatically to significantly affect WBC adhesion and transvascular exchange of fluid. Supported by NIH R01 HL-39286.

S1D
AN UPDATE ON FREE RADICALS
KE Arfors
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Phagocytosing inflammatory cells (Polymorphonuclear Leukocytes, Macrophages and Monocytes) produce free radicals during the "oxygen burst" and are an example of how controlled free radical generation may be important in bacterial killing. It has been suggested that free radicals derived from oxygen play an important role in the pathogenesis of damage induced by irradiation, inflammation and ischemia.

Free radicals generated by metabolism, pollution, inflammation and/or stress are with increasing age increasing hydrogen peroxide (H_2O_2) which is long-lasting and can move in and out of cells. This is partly explained by decreasing catalase which occurs with increasing age but there is also and underperformance of other antioxidants.
Normal older laboratory mice at an age of 15 months of a lifespan of 2 years will start to dys-regulate cytokines, they form IL6, IL12 and later IL10 which will down-regulate the immune system. NF□B is activated by H_2O_2 and just increasing their level of vitamin E will normalize their cytokine dys-regulation. If vitamin E is removed in diet, dys-reulation starts already after 5-6 months (Daynes, Raymond A Salt Lake City, Utah)
Now "Duling's weed" has been accepted as Glycocalyx and is defined in recent work by Hans Wink and others. We will compare events in the microcirculation with our old data on free radical generation and inhibition with scavengers as SOD, catalase. With the modern ideas of Glycocalyx. Also ischemia reperfusion is following similar routes. Damages in the microcirculation could be inhibited by substances like superoxide dismutase and other scavengers.
I am now suggesting that carbohydrates in our diet are generating free radicals that damage the lining of the microcirculation to create chronic inflammation that leads to heart/ vascular, stroke and diabetes.

Tuesday, August 26th
Lymphatic functional biology: new insights I.
Parallel Symposium – S2

S2A

HYDRODYNAMIC REGULATION OF LYMPHATIC PUMP FUNCTION

DC Zawieja, OY Gasheva, Z Nepiyushchikh, W Wang, M Muthuchamy, AA Gashev
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Hydrodynamic forces are important intrinsic regulators of lymphatic pump function. Lymphatics react to changes in transmural pressure and luminal shear stress, but the responses to these hydrodynamic forces differs dramatically. In response to pressure elevation, lymphatics increase their phasic contraction frequency, initially increase then at higher pressures decrease the strength of the phasic contractions, with reductions in tone. High, steady shear profoundly inhibits lymphatic contractile activity (both phasic and tonic) and reduces contraction frequency, while the low oscillatory shears generated by lymph pump flow enhance phasic contraction amplitude. Additionally, the magnitude and sensitivity of these contractile responses are variable in lymphatics from different body regions. We believe this variability is related to the chronic adaptations by these vessels to inter-regional differences in pressure and shear. To test these hypotheses, we studied isolated lymphatics from different regions of the lymphatic tree in which the hydrodynamic forces vary. Methods we used to study the regulation of lymphatic pump function by these hydrodynamic forces include; isolated/cannulated vessels with controlled pressure/flow, mRNA and protein analyses. We conclude that hydrodynamic forces are intrinsic regulators of lymphatic pump function and that the mechanisms by which lymphatic pumping is regulated are critical issues to be studied.
Supported by NIH HL070308, HL75199 and HL80526.

S2B

ACTIVE REGULATION OF LIPID TRANSPORT AND METABOLISM BY LYMPHATICS: COMPLIMENTARY IN VIVO AND IN VITRO STUDIES

JM Rutkowski [1], JB Dixon [1], V Angeli [2], H Wiig [3], MA Swartz [1]
[1] Inst. of Bioengineering, École Polytechnique Fédéral de Lausanne (EPFL), Lausanne, Switzerland, [2] Immunology Programme, National Univ. of Singapore, Singapore, [3] Dept. of Biomedicine, Univ. of Bergen, Norway.

Lipid uptake and transport by lymphatic vessels are critically important functions, but the active regulation of lipid uptake by lymphatic vessels has not been described, and the potential for lymphatic vessels to affect not only lipid transport, but also lipid metabolism, remains unexplored. We examined these phenomena in complementary in vivo and in vitro models. First, we demonstrate that dysfunctional lymphatic transport leads to significant tissue lipid accumulation as well as altered lipid metabolism and increased cholesterol, suggesting that lymphatic dysfunction may have systemic consequences. Conversely, in the dyslipidemic ApoE null mouse, we found the lymphatic capillaries to be significantly hyperplastic with age, resulting in quantitatively decreased uptake function. Thus, we have evidence of both lymphatic dysfunction negatively regulating lipid metabolism, as well as dyslipidemia negatively affecting lymphatic function. To explore active mechanisms, we developed an in vitro model to recreate the intestinal lacteal microenvironment and quantitate chylomicron transport through enterocyte-ECM-lymphatic cocultures. We use this to show the importance of lymphatic endothelium on regulating chylomicron secretion and to explore mediators of this crosstalk. This work highlights the potentially far-reaching implications of the interplay of lymphatic function and lipid metabolism.

S2C

THE INTER-RELATIONSHIP BETWEEN INTERSTITIAL FLOW, LYMPHANGIOGENESIS, AND THE EXTRACELLULAR MATRIX

J Goldman, E Ongstad, J Uzarski, S Gibbs, L Spaude
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Lymphangiogenesis is considered a promising approach for increasing fluid drainage during secondary lymphedema. However, organization of lymphatics into functional capillaries may be dependent upon pre-existing interstitial flow (IF) and IF may be dependent upon the reformed extracellular matrix at the site of injury. We compared lymphangiogenesis in regenerating skin with normal or reduced IF. It was found that reduced IF diminished lymphatic migration and functional capillary regeneration. We then created a lymphatic obstruction that produces lymphedema in mouse tail skin. IF and lymphedema resolution occurred across the scar free obstruction but were inhibited when the obstruction formed into a scar. VEGF-C therapy ameliorated lymphedema across the scar free wound site but not across the obstructive scar. Neutralization of either VEGFR-2 or VEGFR-3 prevented lymphangiogenesis, but neither receptor neutralization inhibited IF or lymphedema resolution. However, combined VEGF receptor neutralization impaired extracellular matrix regeneration at the wound site and inhibited lymphedema resolution. These findings suggest that resolution of lymphedema in the mouse tail skin may be more dependent upon IF and regeneration of the extracellular matrix across the obstruction than lymphatic capillary regeneration and that VEGF-C may exert its therapeutic effects via increased combined VEGFR-2 and VEGFR-3 signaling.

S2D

THE LYMPH MICROCIRCULATION AND SHOCK

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The microcirculatory disturbance theory of shock mainly focuses on the blood microcirculation disturbances. However, to fully understand the pathogenesis of shock and put forward new strategies of prevention and therapy for shock, it is necessary to reveal the role of lymphatic microcirculation played in shock. A series of researches on hemorrhagic shock, anaphylactic shock, endotoxic shock, trauma hemorrhagic shock and SMAO shock in rat, dog and sheep have been carried out, and proved that lymphatic microcirculation plays a compensatory role and contributes to reversion of shock on the early stage; On the end stage of shock, owing to the increase in the permeability of intestinal mucosa and failure of intestine barrier, bacterial endotoxin and other bioactive compound reflux take place through mesenteric lymphatics, which leads to MODS. The lymphatic microvessel has a structure with abundant opening conjunctions and owes capability to absorb biomacromolecule, which determines that lymph fluid should play more important role on the pathogenesis of MODS than portal vein blood does. These results throw new light on understanding the pathogenesis of severe hemorrhagic shock, and also put forward new strategies of prevention and therapy for shock. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

Tuesday, August 26th
Metabolism and redox changes-ion channel function in pulmonary artery and regulation of pulmonary circulation
Parallel Symposium – S3

S3A

CURRENT PERSPECTIVES ON THE ROLE OF REDOX IN HYPOXIC PULMONARY VASOCONSTRICTION

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Hypoxic pulmonary vasoconstriction (HPV) is an adaptive mechanism that allows optimal matching of pulmonary perfusion to ventilation. The mechanisms of HPV reside in the vasculature, but their nature remains controversial. Whilst it is generally accepted that mitochondria act as the oxygen sensor, there is little consensus as to distal signalling pathways. There are currently three favoured hypotheses, two of which involve redox signalling; all are based on the supposition that oxygen limitation slows the electron transport chain (ETC). The Redox hypothesis proposes that the latter leads to a more reduced cytosol and decreased generation of reactive oxygen species (ROS), causing inhibition of K_V channels, depolarisation and voltage-dependent Ca^{2+} entry. In direct contrast, the ROS hypothesis proposes that a paradoxical increase in mitochondrial ROS generation acts as the signalling moiety, with effectors including voltage-independent Ca^{2+} entry, Ca^{2+} release from ryanodine sensitive stores, and Rho kinase-dependent Ca^{2+} sensitisation. The AMP kinase hypothesis however does not involve redox, but instead a rise in AMP/ATP ratio, which activates AMP kinase leading to cADP ribose-induced Ca^{2+} release from ryanodine-sensitive stores. Current evidence for and against these hypotheses will be discussed, with the conclusion that the ROS hypothesis is most consistent with currently available data. Work in the Author's laboratory is supported by the Wellcome Trust and British Heart Foundation

S3B

REDOX REGULATION OF ION CHANNELS AND PULMONARY ARTERY FUNCTION IN HPV

AL Cogolludo
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Inhibition of voltage-gated potassium channels (Kv channels) has been shown to contribute to hypoxic pulmonary vasoconstriction (HPV), a physiological mechanism that optimizes blood oxygen saturation by increasing pulmonary vascular resistance in poorly aerated lung regions. Herein, we have examined the mechanisms involved in the vasoconstriction and Kv channel inhibition following acute hypoxia in resistance pulmonary arteries. We found that hypoxia increased ceramide content measured by immunocytochemistry, phosphorylation of the membrane NADPH oxidase subunit $p47^{phox}$, reactive oxygen species production measured by either dihydroethidium or dichlorofluorescein fluorescence, inhibited Kv currents and induced a vasoconstrictor response in isolated rat pulmonary arteries (PA). Inhibition of NAPDH oxidase, protein kinase C (PKC), neutral sphingomyelinase (nSMase) abrogated the changes in reactive oxygen species, Kv currents and the vasoconstrictor response. nSMase inhibition also inhibited the increase in ceramide and prevented HPV in anesthetized open-chest rats. The signaling pathway was specific for small PA as compared to mesenteric arteries. nSMase2 mRNA expression was 10 fold higher in small PA compared to mesenteric arteries. In conclusion, nSMase-derived ceramide production and activation of PKC are early and necessary events in the signaling cascade of HPV leading to increased reactive oxygen species production, inhibition of K_V channels and vasoconstriction. Supported by SAF2005-03770 and Mutua Madrileña.

S3C

GLUCOSE METABOLISM, REACTIVE OXYGEN SPECIES (ROS), AND REGULATION OF PULMONARY ARTERY FUNCTION

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It is well known that pulmonary artery (PA) function is regulated by changes in alveolar oxygen tension (pO_2) to maintain perfusion-to-ventilation ratio in the lungs. However, the O_2 sensor and mechanism(s) that regulate function of PA by change in pO_2 remain elusive. Although there is consensus that ROS are involved in signaling; there are two opposite views, and it is unclear as to ROS is increased or decreased by hypoxia. One hypothesis proposes inhibition of mitochondrial respiratory chain by hypoxia increases ROS production, which induces hypoxic pulmonary vasoconstriction (HPV). In contrast, redox hypothesis proposes that a decrease in ROS by hypoxia lowers cGMP and inactivates K_v channels leading to PA constriction and HPV. Recently, we have demonstrated that activation of glucose-6-phosphate dehydrogenase (G6PD), a rate limiting enzyme in the pentose phosphate shunt, by hypoxia increases NADPH-to-$NADP^+$ ratio in PA and lungs, and elevation in cytosolic NADPH is associated with the development of HPV. Consistently, inhibition of G6PD reduces HPV and ameliorates hypoxia-induced pulmonary hypertension in rats. As NADPH redox can regulate ion channel function and NADPH oxidase, we propose changes in G6PD activity and NADPH redox could play a key role, directly or indirectly *via*

ROS, in the development of HPV and PH. This work is supported by American Heart Association (0435070N) and NIH (R01HL085352).

S3D

THE ROLE OF RADICALS IN HYPOXIC PULMONARY HYPERTENSION (HPH)

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HPH develops in rats within the first days of exposure to hypoxia and than it stabilizes. Pathogenesis of HPH is initiated by radical injury to pulmonary vascular wall. To test timing of radical release we measured in rats the lung release of NO, superoxide and plasma concentration of nitrotyrosine after 1, 4 and 21 days of hypoxia. The lipid peroxidation was assessed by concentration of lipofuscin - like pigments (LFP) in erythrocytes. In vivo NO production in expired air was highest at 1st and 4th day of hypoxia and it significantly decreased but not normalized at day 21. In isolated lungs the NO (NOx accumulation in perfusate) peaked at the 1st day of hypoxia and preceded the peak of O_2^- production (4th day). After 21 days of hypoxia NO and O_2^- production did not differ from normoxic controls. Concentration of nitrotyrosine increased 1st day, LFP 4th day and both stayed high till 21st day of hypoxia. Histochemically we observed in prealveolar vessels of normoxic and hypoxic rats the presence of O_2^-, while H_20_2 occurred in hypoxic rats only. We conclude that increased lung radical formation is present at the beginning of HPH and does not continue in later phases of exposure.

Tuesday, August 26th

Frontiers in cardiac microcirculation: stem cells and the endothelium. Do stem cells improve cardiac microcirculation?

Parallel Symposium – S4

S4A

ENDOTHELIAL FUNCTION AND ANGINA

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Normal or non-obstructive coronary disease at angiography is not uncommon and occurs in 10 % of women presenting with ST-segment elevation myocardial infarction compared to 6% in men. Patients with evidence of myocardial ischemia/myocardial infarction and non-obstructive coronary arteries are more likely to be women and non-white. Among this subgroup with evidence of myocardial damage, 50% have no visible disease by coronary angiography. Prognosis for patients with unstable angina and non-obstructive coronary artery disease is not benign, and includes 2% of death and myocardial infarction at 30 days follow-up. New studies demonstrate that at least 20% of women with normal or non-obstructive angiography have myocardial ischemia, likely due to vascular dysfunction, which is associated with adverse cardiac events. Among women with normal coronary angiography, the presence of vascular dysfunction in the form of endothelial dysfunction is associated with a 30% rate of future obstructive coronary artery disease development over the next years. Endothelial dysfunction with non-obstructive coronary arteries also has an adverse prognosis, with a 4-year event rate of up to 14%. Randomized placebo-controlled studies have demonstrated that tricyclics, beta-blockers, ACE-inhibitors, L-arginine and statins may relieve symptoms and /or vascular dysfunction, however longer-term studies evaluating cardiac event rates need to be performed.

S4B

DRUGS MODULATING ENDOTHELIAL FUNCTION

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Atherosclerosis is a chronic systemic disease of the vasculature with an inflammatory component. Several studies have demonstrated a strong association between coronary vascular endothelial dysfunction and an adverse long-term cardiovascular prognosis. Both conduit arterial and microcirculatory endothelial function are predictive of outcome independent of the presence of coronary artery disease and its risk factors. Usually, vascular immune responses precede development of vasodilator dysfunction. A reduced bioavailability of nitric oxide (NO), an alteration in the production of prostanoids, including prostacyclin, thromboxane A2, and/or isoprostanes, an impairment of endothelium-dependent hyperpolarization, as well as an increased release of endothelin-1, can individually or in association contribute to endothelial dysfunction. Modalities to interfere with vascular inflammation and endothelial function include physical exercise, cessation of smoking, and weight reduction as important lifestyle changes; furthermore certain antioxidants like flavonoids, antihypertensives, inhibitors of the renin-angiotensin system, statins, endothelin-antagonists, and treatment of hyperglycemia with diet or using antidiabetic drugs ameliorate impairment of endothelial cell function. In contrast, specific immunosuppressive drugs may disturb endothelial function. Whether strategies that improve endothelial function will uniformly improve prognosis needs to be studied prospectively.

S4C

GROWTH AND FUNCTION OF HUMAN BLOOD VESSELS FROM VASCULAR PROGENITOR CELLS (VPC) IN THE DOG HEART WITH A CRITICAL STENOSIS OF THE LEFT ANTERIOR DESCENDING CORONARY ARTERY.

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We have previously identified and characterized adult cardiomyocyte progenitor cells from the human heart. The goal of the present study was to isolate, expand and determine the potential of VPCs in the treatment of myocardial ischemia in the dog heart. Human coronary vascular progenitor cells (hVPC) were isolated and transfected with GFP. The cells were expanded and injected (10^6 cells) into the apical wall of the dog heart in the area of a critical stenosis in the hope of generating a collateral circulation. Dogs were immunosuppressed with cyclosporine. One month later hVPC derived blood vessels were evident by GFP fluorescence of smooth muscle and endothelial cells in the area of the LAD stenosis and consisted of blood vessels with diameters ranging from 50 um to 1.5 mm, some containing red blood cells. The human ALU antigen was evident by confocal microscopy indicating that these vessels were derived from hVPC. At one month after hVPC injection, upon total occlusion of the LAD coronary artery in the conscious dog, microsphere measured blood flow to the area of chronic ischemia was increased above that of normal levels. The vessels were also visualized during coronary angiography and three patterns were recognized during coronary occlusion: 1) filling of the LAD which then dissipated

slowly, 2) filling of what appeared to be a newly developed large epicardial (1.5 mm) coronary artery, and 3) appearance of an epicardial halo creating a shadow on the epicardial surface. Thus, hVPC administration in a large animal heart results in the formation of functional large and small human coronary blood vessels which enhance blood flow in the area of a critical coronary stenosis and suggest that the use of VPCs has great potential in the treatment of myocardial ischemia especially where bypass surgery is not possible.

Tuesday, August 26th
Recent advances in laser doppler flowmetry of the cutaneous microcirculation
Parallel Symposium – S5

S5A
LASER DOPPLER FLUXIMETRY AND PERFUSION IMAGING: OPPORTUNITIES AND PITFALLS.

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Laser Doppler techniques offer exciting opportunities to study the microcirculation in both research and clinical settings. They are simple to use and robust. Accurate interpretation of the results is however dependent upon appropriate experimental design, attention to detail and an understanding of the anatomy and physiology of the organ under investigation. When used with scientific rigor these techniques demonstrate important new aspects of microcirculatory function.
Physiological assessments, eg reactive hyperaemia, are important tools. With rigorous assessments of the perfusion profile which occurs on release of the occlusion, 3 different profiles have been identified. An early dominant peak in the response (before 2 secs), considered to be the most "abnormal" response, is associated with a more at risk cardiovascular phenotype. In combination with other techniques, eg iontophoresis or microinjection, laser Doppler techniques are used to assess endothelial dependent and independent vasodilatation, or the vasoactive effects of peptides or hormones. When used with antagonists of components in the proposed pathway they allow dissection of the pathways involved in health and disease states. Laser Doppler technologies are also being used to assess long term vascular responses following therapeutic interventions or lifestyle interventions. Such investigations place additional burdens on the technology. Methods to ensure that the instruments being used are robust enough for such approaches will be discussed.

S5B
THE INVESTIGATION OF SKIN BLOOD FLOW MOTION IN VASCULAR DISEASES

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Skin blood flow motion is the cyclical variation in skin blood flow due to the rhythmic contraction and dilation of skin arterioles, the so called vasomotion. Vasomotion and the consequent blood flow motion have been suggested to be important factors in the optimisation of the blood flow distribution in microcirculatory beds. They can be investigated in clinical setting by means of laser Doppler flowmetry. The same method combined with spectral analysis of laser Doppler signal allows the indirect investigation of the different mechanisms involved in the control of vasomotion, such as the endothelial activity, the local sympathetic activity and the spontaneous activity of microvessel smooth muscle cells. Using this method, a significant amplification in skin flow motion was observed during post-ischemic re-perfusion in healthy subjects. On the other hand, a blunted or a completely absent post-ischemic increase in skin flow motion has been found in patients with different pathological conditions, such as arterial hypertension, peripheral arterial obstructive disease, systemic sclerosis, diabetes, obesity, hypercholesterolemia or chronic renal failure, as well in smokers. These findings suggest that spectral analysis of skin laser Doppler signal can be a useful method to detect skin microvascular impairment in vascular diseases and to investigate the mechanisms involved in this impairment.

S5C
LASER-DOPPLER FLOWMETRY AND IN VIVO PHARMACOLOGY IN HUMAN SKIN MICROCIRCULATION

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The studies of microvascular function in human skin can be performed using laser-Doppler (LD) flowmetry. This method enables wide range of application of vasoactive substances, with their advantages and faults. At the present, different local or systemic administration techniques are in use. The disadvantage of systemic use of drugs is that we are not able to exclude the systemic effects that indirectly influence microvascular flux response. For this reason, the local application is preferred. The ionthophoresis is method of choice for local non-invasive application of substances. However, it is restricted only to small charged molecules. Drug delivery patches may only be used with small lipid-soluble drugs. For all other types of substances, only minimally invasive techniques could be employed. The microdialysis has to be applied with the use of anaesthesia that influences LD flux. The doses of drugs used with this method must be high and might therefore produce side-effects. In contrast, the microinjection requires small quantities of substances. The disadvantage of last technique is necessity of double injection technique with application of saline solution as a control. However for now, all these methods are only applicable in vivo pharmacology in human skin microcirculation in health and disease.

S5D
REGIONAL VARIATIONS IN THE RHYTHMIC COMPONENTS OF THE HUMAN CUTANEOUS MICROCIRCULATION

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Low-frequency, spontaneous, rhythmic variations (vasomotion) in cutaneous blood flow (CuBF) with dominant frequencies ranging from 1.5 to 20 cycles/min (cpm,) were identified in the human skin using laser-Doppler flowmetry (LDF). We systematically investigated the vasomotion in 15 different skin regions by LDF, and tested the temporal stability of the spectral parameters as compared with the determination of post-occlusive hyperemia of the forearm skin. LDF data were recorded 4 times (0, 1, 12 and 24 months) in healthy volunteers (20-22 years, n=14) as described (1). Vasomotion was found in 11 skin regions. The dominant frequency of vasomotion significantly decreased from the midline to the more lateral skin areas, (mean±SEM, *p<0.05) from 7.9±0.3 (forehead) to 5.8±0.5* cpm (lateral thigh). Characteristics of vasomotion did not change over time (e.g.: on the templates 8.4±0.3, 8.3±0.4; 8.4±0.4, 8.3±0.4 and 8.2±0.4, 8.3±0.3, cpm at 0, 12 and 24 months, respectively). In contrast, characteristic parameters derived from CuBF values of reactive hyperemia were highly variable during the repeated challenges. Our data indicate that the oscillations in the microcirculation exhibit a regional-dependent pattern, which seems largely time-invariant whereas the parameters of reactive hyperaemia show poor solidity.
Ref.: (1) Microvasc Res 70:121-8, 2005. Supported by OTKA (K63401).

S5E

MICROVASCULAR DYSFUNCTION: A POTENTIAL MECHANISM IN THE PATHOGENESIS OF OBESITY-ASSOCIATED INSULIN RESISTANCE AND HYPERTENSION

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The metabolic syndrome defines a clustering of metabolic risk factors, which confers an increased risk for type 2 diabetes and cardiovascular disease. The metabolic syndrome seems to have multiple etiological factors and microvascular dysfunction may be one potential factor explaining the clustering of multiple metabolic risk factors including obesity, hypertension, insulin resistance and glucose intolerance. Recently, it has become clear that obesity is characterized by microvascular dysfunction, which may increase not only peripheral vascular resistance but also decrease insulin-mediated glucose disposal in muscle, thereby contributing to hypertension and insulin resistance, respectively. Whereas assessment of microvascular function of skeletal muscle would be most relevant from a pathophysiological standpoint, it requires invasive and complicated techniques. We postulated that the cutaneous circulation may be a representative vascular bed to examine the mechanisms of microvascular dysfunction, which may mirror generalized systemic vascular dysfunction in magnitude and underlying mechanisms. Making use of videomicroscopy and laser Doppler fluxmetry combined with iontophoresis, we assessed the role of microvascular dysfunction as a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension.

Tuesday, August 26th
Molecular diversity of VEGF-A
Parallel Symposium – S6

S6A
MOLECULAR DIVERSITY OF VEGF-A
DO Bates
Microvascular Research Laboratories, Dept. of Physiology and Pharmacology, School of Veterinary Sciences, Univ. of Bristol, Bristol, UK.

Vascular Endothelial Growth factor – A is alternatively spliced to form multiple isoforms with differing properties. Alternative splicing of exons 6 and 7 results in isoforms with differing heparin binding and Neuropilin activation, whereas alternative splicing of exon 8 results in isoforms with different angiogenic properties. The inclusion of intron 7 results in greater binding to both heparin and neuropilin, whereas exon 6 inclusion gives even stronger heparin binding. This results in altered bioavailability and spatial cues during growth of new vessels. Choice of either a proximal or distal splice site in exon 8 results in two families of proteins –the $VEGF_{xxx}$ angiogenic splice isoforms resulting from proximal splice site selection, or the $VEGF_{xxx}b$ family resulting from distal splice site selection. These two families are regulated separately, and have different functions in vivo. A balance between the two isoforms is increasingly being demonstrated as being essential for normal vascular maintenance. $VEGF_{xxx}b$ isoforms have now been shown to be anti-angiogenic, but also allow revascularisation of ischemic tissues. The evidence for the mechanisms of actions, control of splicing and physiological function of the different VEGF-A isoforms will be discussed in this symposium.

S6B
REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGFA) ISOFORMS MAY BE A MECHANISM TO REGULATE SEX-SPECIFIC VASCULAR DEVELOPMENT, CORD FORMATION AND FOLLICLE PROGRESSION WITHIN DEVELOPING GONADS.
AS Cupp, RC Bott, R Pohlmann, RAT Broeck, DT Clopton
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My laboratory is investigating the role of VEGFA in the development of sex-specific vascular and gonadal structures in the male and female rodent. The *Vegfa* gene contains 8 exons and is alternatively spliced to produce pro-angiogenic (*Vegfaxxx)* and anti-angiogenic isoforms (*Vegfaxxxb*). VEGFA binds to its receptor Kinase Domain Region Receptor (KDR) to direct neovascularization of tissues. The testis differentiates earlier than the ovary and cord formation and sex-specific vasculature are hallmarks of its development. Inhibition of VEGFA in testis organ cultures arrested both cord formation and sex-specific vascular development. However, inhibition of anti-angiogenic isoforms or addition of VEGFA angiogenic isoforms increased vascular development but perturbed cord formation. Using KDR/LacZ mice, we established that pre-endothelial mesonephric cell migration occurs in the testis, but not the ovary, suggesting that vascular origins are sex-specific. Using peri-natal ovarian cultures, we demonstrated that inhibition of VEGFA not only affects vascular development in the ovary but also inhibits follicle progression. Additionally, inhibition of VEGFA anti-angiogenic isoforms or angiogenic VEGFA isoforms induced follicle progression. Thus, regulation of the *Vegfa* gene to produce angiogenic or anti-angiogenic isoforms may be a mechanism to alter sex-specific vascular development, formation of seminiferous cords, and/or follicle progression within mammalian gonads. This research was supported by grants from NIH (HD041546, HD045350 and HD051979) and Nebraska Tobacco Funds for Biomedical Research.

S6C
REGULATION OF SPLICING CONTROLS THE BALANCE OF PRO- AND ANTI-ANGIOGENIC VEGF ISOFORM EXPRESSION.
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Vascular Endothelial Growth Factor (VEGF) exists as two families of sister isoforms formed by differential splice site selection in exon 8. The distally spliced isoforms, $VEGF_{xxx}b$, are anti-angiogenic. Environmental cues activate angiogenesis through increasing VEGF mRNA and protein secretion. In cells expressing both pro- and anti-angiogenic isoforms of VEGF, TGF-□1 dose-dependently stimulated expression of $VEGF_{xxx}b$ using distal splice site selection (via p38 MAP kinase and Clk1, 4). IGF-1 treatment favoured proximal splice site selection; expression of $VEGF_{xxx}b$ in cells incubated with IGF-1 was significantly decreased. The splicing factor, SRp55, also affects the differential splicing of VEGF, favouring $VEGF_{xxx}b$ production. Western blotting analysis showed that SRp55-transfected cells increased $VEGF_{165}b$ expression, whereas two different shRNAis to SRp55 both significantly decreased $VEGF_{165}b$ expression compared to control cells. Moreover, the 35 nt region around the stop codon of $VEGF_{xxx}b$ mRNA shows SRp55 binding activity. The results demonstrate that VEGF isoform expression can be regulated. The switch from anti- to pro-angiogenesis may be a fundamental control mechanism in a number of vascular diseases, as well as in normal development. Understanding this splicing switch will enhance our understanding of blood vessel growth in normal tissues and in pathological states as diverse as diabetes and cancer. (Supported by the Wellcome Trust)

S6D

ACTIVATION OF RECEPTOR TYROSINE KINASES: VEGFR-2/ NEUROPILIN-1 CO-RECEPTOR COMPLEX FORMATION BY DISTINCT VEGF ISOFORMS.

S Cébe-Suarez [1,6], FS Grünewald [2], R Jaussi [2], X Li [3], L Claesson-Welsh [3], D Spillmann [4], AA Mercer [5], AE Prota [2], K Ballmer-Hofer [1]

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Vascular endothelial growth factors, VEGFs, interact with several types of receptors, receptor tyrosine kinases (RTK) such as VEGFR-1, -2, and -3, neuropilins such as NRP-1, and heparansulfate glycosaminoglycans (HS). VEGF RTKs signal to downstream targets upon tyrosine phosphorylation mediated by the activated intracellular kinase domain. NRP-1, on the other hand, has no enzymatic activity itself but apparently modulates signal output by VEGF receptors. Finally, HS enhances signaling by VEGF receptors presumably by concentrating VEGF in the extracellular matrix adjacent to cells thereby augmenting receptor binding. VEGFs are produced in several isoforms generated by alternative splicing and proteolytic processing. A variety of VEGF-As and orf virus variants, VEGF-Es, have been characterized to date, all of them showing potent angiogenic activity in vitro and in vivo. Specific splice variants of VEGF-A, which differ in the last six amino acids at the carboxyterminal end, have been shown to lack NRP-1 binding and to display attenuated signaling in endothelial cells.

Here we determined the exact NRP-1 binding domain of VEGF-A and -E. Comparing these two VEGFs allowed us to distinguish between NRP-1 binding in the absence (VEGF-E NZ2) and in the presence (VEGF-A165) of an HS binding sequence. The carboxyterminal peptide of VEGF-E, RPPR, showed high affinity for NRP-1, while the peptide DKPRR, encoded by exon 8 of VEGF-A, bound NRP-1 only with low affinity. A chimeric VEGF in which RPPR replaced the authentic carboxyterminus of VEGF-A121, showed that this peptide was both necessary and sufficient for NRP-1 binding. Upon stimulation of cells with VEGF, VEGFR-2 and NRP-1 formed stable coreceptor complexes independent of whether the receptors were expressed on the same or on separate cells. The short form of VEGF-A, VEGF-A121 which lacks a HS binding sequence, was unable to promote coreceptor complex formation, while the long form, VEGF-A165, as well as VEGF-E NZ2, efficiently promoted coreceptor assembly. Signal output by VEGFR-2 in the presence or absence of NRP-1 was different and microvessel formation was severely impaired in tissues treated with VEGFs deficient for NRP-1 binding. Taken together, our data suggest that angiogenic signaling by VEGFR-2 critically depends on the presence of NRP-1 in a multimeric coreceptor complex. These findings open new clinical possibilities for the development of therapies aimed at interfering with angiogenesis in disease.

PL2
CARDIOVASCULAR FUNCTIONS OF THE ENDOCANNABINOID SYSTEM

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Endocannabinoids (ECs) are ubiquitous lipid mediators generated in the cell membrane that activate the same receptors that recognize the bioactive marijuana constituent delta-9-tetrahydrocannabinol (THC) and produce similar effects. The two best characterized ECs are anandamide (arachidonoyl ethanolamide) and 2-arachidonoylglycerol (2-AG), which can interact with CB1, CB2 and additional as yet undefined receptors. In rodents, activation of CB1 receptors by ECs, THC or its synthetic analogs can elicit profound and long lasting hypotension, suggesting EC involvement in hypotensive states. Indeed, macrophage- and platelet-derived endocannabinoids have been implicated in the hypotension associated with various forms of shock, including hemorrhagic, endotoxic, cardiogenic shock and the hypotension associated with advanced liver cirrhosis, with potential therapeutic value of CB1 blockade. In normal, healthy animals, there is no EC-mediated hypotensive tone, but the EC system becomes tonically active in various forms of hypotension and acts as a compensatory mechanism to maintain blood pressure at physiological levels. Treatment of hypertensive rats with synthetic inhibitors of fatty acid amidohydrolase, the enzyme responsible for the in vivo degradation of anandamide, normalizes blood pressure and the inappropriately increased cardiac contractility, suggesting that targeting the EC system may have therapeutic value in the treatment of hypertension and the associated cardiac hypertrophy.

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Dr. Kunos received his M.D. degree in 1966 from Semmelweis University in Budapest and his Ph.D. in 1973 from McGill University in Montreal where he worked with Mark Nickerson on adrenergic receptors. In 1974 he joined the faculty in Pharmacology and Medicine at McGill University and then in 1987 joined the NIAAA as Chief of the Laboratory of Physiologic and Pharmacologic Studies. Dr. Kunos moved to the Medical College of Virginia in 1992 to Chair the Department of Pharmacology & Toxicology. In 2000 he returned to NIH as Scientific Director of NIAAA. He is an elected fellow of the High Blood Pressure Research Council of the American Heart Association and a foreign member of the Hungarian Academy of Sciences.

Dr. Kunos' laboratory is studying the role of endocannabinoids in neuroendocrine and cardiovascular regulation. We have identified a paracrine mechanism whereby endocannabinoids generated in macrophages and platelets act on vascular cannabinoid receptors to elicit vasodilation. We are exploring the role of this mechanism in the regulation of vascular tone, the factors that activate this pathway, the signaling pathways involved in both the generation and the action of endocannabinoids, and the identity of as-yet-unidentified vascular cannabinoid receptor(s). We use physiological approaches in whole animals or isolated tissues, as well as cell and molecular biological techniques.

Cannabinoids increase appetite and our laboratory has provided evidence that endocannabinoids acting at CB1 receptors in the brain are involved in mediating the hunger-induced increase in food intake. We are interested in identifying the nature of the interactions between endocannabinoids and other hormones and neuropeptides implicated in the neural control of appetite, as it affects food intake as well as alcohol drinking behavior. We use a combination of behavioral, physico-chemical (mass spectrometry) and immunohistochemical approaches.

A third are of interest relates to the molecular mechanisms involved in liver regeneration. We are interested in the role of cytokines and other mediators generated by Kupfer cells in liver regeneration and in alcohol-induced liver damage.

Wednesday, August 27th
Vascular damage in hypertension. insights form basic and clinical research
Parallel Symposium – S7

S7A

SMALL ARTERIES, PERIPHERAL VASCULAR RESISTANCE AND ORGAN DAMAGE IN HYPERTENSION

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The hallmark of hypertension is an increase in peripheral vascular resistance. Studies of small arteries have consistently demonstrated that there is a decrease in internal lumen diameter with an increase in media wall thickness but no change in cross-sectional area and that the normal response to high blood pressure is termed eutrophic inward remodelling. This appears to occur as a result of the increase in wall stress, which a rise in blood pressure must bring about. At this level of the circulation, one of the primary functional roles of small arteries is to regulate the flow of blood to downstream tissues. Much of this is facilitated by the myogenic reflex. When this is defective as is often observed in diabetes or in fulminant hypertension, there is an increase in wall stress and the compensatory mechanism for eutrophic remodelling is inadequate and is replaced by a growth response and hypertrophy of the vascular wall. Studies most recently have demonstrated in a variety of tissues that the development of vascular wall hypertrophy is associated with target organ damage. In a series of studies recently completed, we have demonstrated that effective management can reverse this process and restore normal vascular integrity. This presentation will look at the molecular mechanisms responsible for eutrophic remodelling, the pugative genetic background to the myogenic response and the fact that pressure independent breakdown of autoregulation may also predispose individuals to particular consequences of hypertension such as stroke and heart disease.

S7B

MICRO- AND MACRO-CIRCULATION IN HYPERTENSION: A VICIOUS CIRCLE THAT SHOULD BE BROKEN BY TREATMENT

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The damaging effect of local pulse pressure (PP) has been well demonstrated on large arteries, and to a lesser extent on small arteries. Elevated PP can stimulate hypertrophy, remodeling, or rarefaction in the microcirculation, leading to increased resistance to mean flow. Recent studies showed a close relationship between microvascular damage in brain and kidney and either pulse pressure or arterial stiffness. Indeed, significant and independent relationships have been demonstrated between carotid stiffness and glomerular filtration rate (GFR) in patients with mild to moderate chronic kidney disease, between brachial pulse pressure and GFR in elderly patients with never-treated isolated systolic hypertension, and between arterial stiffness and cognitive impairment in elderly subjects attending a geriatric outpatient clinic. The damage of cerebral and renal small arteries can be explained on the basis of differential input impedance in the brain and kidney compared with other systemic vascular beds. The unique features of the kidney and brain are that they are continually and passively perfused at high-volume flow throughout systole and diastole. Their vascular resistance is very low, so that in comparison to other vascular beds, resistance is closer to input and characteristic impedance. Torrential flow and low resistance to flow in these organs expose small arterial vessels to the high-pressure fluctuations that exist in the carotid, vertebral, and renal arteries. Such fluctuations, measurable as central pulse pressure, increase 3- to 4-fold with age. Exposure of small vessels to highly pulsatile pressure and flow may explain microvascular damage and resulting renal insufficiency and intellectual deterioration. A vicious circle between small and large artery damage can be exemplified by the following: (a) increased wall-to-lumen ratio and rarefaction of small arteries are major factors for an increase in mean BP; (b) the higher mean BP in turn increases large artery stiffness, through the loading of stiff components of the arterial wall at high BP levels; (c) the increased large artery stiffness is a major determinant of the increased PP, which in turn damages small artery, as seen above, and favours the development of left ventricular hypertrophy, carotid intima-media thickening and plaque rupture. These various types of target organ damage have been shown to be related with CV events. Antihypertensive treatment should break this vicious circle, to increase the possibilities of reversing target organ damage and reducing the risk of CV events. Not all pharmacological classes are equal in this respect. ACE inhibitors, angiotensin II receptor antagonists, diuretics and calcium channel blockers have been repeatedly shown to reduce arterial stiffness, central BP and augmentation index, and small artery damage. By contrast, nonvasodilating beta-blockers have opposite effects, i.e. they increase central BP and augmentation index and do not reduce small artery damage. These pharmacodynamics data can be paralleled by large outcome clinical trials. In the LIFE and ASCOT studies, losartan- and amlodipine-based treatments, respectively, proved to be more effective than atenolol-based treatments for reducing CV events. Taken together, these data also suggest that therapy based on brachial artery recordings may overestimate the effect of beta-blocking drugs on central aortic systolic BP and underestimate the effectiveness of ACE inhibitors, angiotensin II receptor antagonists, diuretics and calcium channel blockers.

S7C
ENDOTHELIAL DYSFUNCTION IN HYPERTENSION

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Healthy endothelium plays a central role in cardiovascular control. Therefore, endothelial dysfunction (ED) may have a particularly significant role in the pathogenesis of atherosclerosis. ED is a consequence of the harmuful effects of risk factors of atherosclerosis on the vessel wall, promotes progression of atherosclerosis and probably plays an important role in the development of thrombotic complications in the late stages of the disease. As ED is a key underlying factors in the atherosclerotic process, markers of endothelial abnormalities have been sought, particularly those involving disturbed endothelium-dependent vasomotion. A large body of evidence indicates that endothelial dysfunction is a characteristic of patients with essential hypertension. Endothelial dysfunction associated with essential hypertension is characterized by impaired NO availability, determined by oxidative stress which causes NO breakdown. Further, an interaction between NO-system and endotelin-1 can participate in the pathogenesis of endothelial dysfunction. Although plasma levels of endothelin-1 do not seem to be increased in essential hypertension, it is worth noting that the vasoconstricotr activity has been found to be increased in parallel with diminished NO availability. In patients with essential hypertension endothelium-dependent vasodilation is impaired to acetylcholine and bradykinin application. These findings indicate that the endothelial dysfunction in this condition is not related to a specific defect of a single intracellular signal-transduction pathway and suggests a more generalized abnormality of endothelial vasodilator function. It is presently unclear whether endothelial dysfunction is a primary abnormality or a consequence of the elevated blood pressure values. Data in animal models are in favour of direct damage of the endothelial cells secondary to a prolonged increase of the haemodynamic load and shear stress. Further, the endothelial dysfunction observed in hypertension appears to be a consequence of high blood pressure since a variety of antihypertensive treatment normalizes this responses. However, in normotensive offsprings of hypertensive parents, an early alteration of endothelium-dependent vasodilation in the forearm was observed. We also found impired andothelial function in offsprings of parents with essential hypertension although their blood pressure was in normal range. These data suggest a possibly relevant role of genetic mechanisms in the genessis of endothelial dysfunction in hypertension. Recent observations also favour the hypothesis that a dysfunctional endothelial NO-synthase gene polymorphism may be associated with essential hypertension, although this was not consistently observed in all populations.
Determination of ED in hypertension has important clinical implications. It was shown that ED is significantly and directly correlated with the presence and intensity of risk factors, and the occurrence of cardiac events and that cardiac events increased as ED worsens.

S7D
MICROVASCULAR DYSFUNCTION IN HUMAN HYPERTENSION

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There are growing evidences that impaired microcirculation plays an important role in the patophysiology of arterial diseases. New data support the role of microcirculation not only in the development of hypertensive target organ damage, but in the pathogenesis of hypertension, as well. Laser doppler flowmetry and capillarmicroscopy give us the possibility to assess microvascular endothelial function and microcirculatory structural changes, which can be observed in hypertension, but also in normotensive offspring of individuals with hypertension. These observations suggest that endothelial dysfunction and capillary rarefaction might play a role in the pathogenesis of primary hypertension. The microcirculation is a key site of increased vascular resistance, which is the major haemodynamic abnormality underlying elevated blood pressure in hypertension. Increase in central aortic stiffness, as measured by carotid-femoral pulse wave velocity, predicts cardiovascular mortality in patients with various cardiovascular diseases independent of other risk factors. It was previously shown, that relation between increased arterial stiffness and impaired microvascular function persisted in models that were adjusted for conventional risk factors, which indicates, that arterial stiffening is accompanied by microcirculatory structural or functional remodeling. We found a significant correlation between parameters of endothelial dysfunction, measured by laser doppler flowmetry and arterial stiffness in hypertensive patients.

Wednesday, August 27th
Extracellular matrix and growth factors
Parallel Symposium – S8

S8A

ANGIOPOIETIN-1 MODIFIES MICROVASCULAR PERMEABILITY COEFFICIENTS VIA AN EFFECT ON ENDOTHELIAL GLYCOCALYX

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We examined Ang1 effects on microvascular hydraulic conductivity (L_P) and reflection coefficient to albumin (sigma) in MS222-anaesthetised frogs using a modified Landis-Michel technique before and after 200 ng/ml Ang1 perfusion. Ang1 decreased L_P (baseline: 2.0+/-0.9 (n=11); Ang1 1.2+/-0.9 (n=11); p<0.05, Wilcoxon) and increased sigma (baseline: 0.78+/-0.03 (n=10); Ang1 0.91+/-0.03 (n=10); p<0.05, paired t-test). Glomerular filtration coefficient (L_PA) and sigma were measured oncometrically *ex vivo* [1-2]. L_PA decreased in Wistar rat glomeruli (baseline: 1.00+/-0.09 (n=30); Ang1 0.76+/-0.06 (n=31); p<0.05, unpaired t-test) and sigma increased in individual mouse glomeruli (baseline: 0.973 (n=15); Ang1 0.980 (n=15); p<0.05, paired t-test) after incubation in 200 ng/ml Ang1. We hypothesised that endothelial glycocalyx modification induced these diverse changes. Ang1-treated human microvascular endothelial cell supernatant decreased 488nm light absorbance by Alcian Blue [0.13+/-0.02 (n=6); vehicle: 0.05+/-0.02 (n=6); p<0.05, unpaired t-test), indicating increased glycosaminoglycan content. Ang1 prevented the pronase-induced mesenteric microvessel L_P fold increase (pronase: 2.6+/-0.4; Ang1 + pronase: 1.2+/-0.3; p<0.05, unpaired t-test) previously attributed to glycocalyx loss. Ang1-induced endothelial glycocalyx modification may explain the Ang1-induced microvascular permeability coefficient changes. Supported by the *Wellcome Trust* and *Kidney Research UK* [1] Salmon et al, *J Physiol* 2006; 570:141-56 [2] Sage et al, *Microcirc* 2007; 14:635-65 (abstract) [3] Adamson *J Physiol* 1990; 428:1-13.

S8B

HYALURONAN SECRETION IS MECHANOSENSITIVE; REGULATORY PATHWAYS *IN VIVO.*

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HA influences interstitial and glycocalyx permeability, conserves synovial fluid and lubricates joints. Its mechanosensitive secretion was studied in joints. Endogenous HA was washed out from cannulated knee-joints of anaesthetised rabbits. Joints were cycled passively at 0-1.5Hz for 0-9min in every 15min for 5h. Newly secreted HA was harvested at 5h for HPLC. Secretion rate *qHA* was a graded, curvilinear function of movement frequency and duration, increasing by 2.1-2.3x (p=0.0001, ANOVA). Transcription-translation inhibitors reduced movement-stimulated *qHA* (MSHA) by 18-30%, as did translocation inhibitor brefeldin. PGE_2 and PKC activator PMA stimulated *qHA* 2-4x. A Ca^{2+}-PKC–MEK-ERK pathway mediates stretch-stimulated *qHA* in culture (Momberger et al 2006 Matrix Biol. 25 306), and Ca^{2+}-ionophore ionomycin doubled *qHA*. But PKC inhibitor bisindolylmaleimide and MEK-ERK inhibitors (U0126, PD98059) did not inhibit MSHA, though they blocked PMA-stimulated secretion. Phospholipase C inhibitor U73122 almost fully blocked movement-stimulated *qHA* (p=<0.001, n=10 pairs). HA secretion is thus a graded function of mechanical stimulation; and phospholipase C (but not PKC-MEK-ERK) is an obligatory step *in vivo.* Mechanosensitive secretion protects tissues from HA depletion, restores lost HA and may contribute to exercise therapy for moderate OA.

S8C

ANGIOPOIETIN-1 REDUCES LOSS OF MICROVASCULAR ENDOTHELIAL INTEGRITY DURING ENDOTOXAEMIA

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Loss of vessel integrity precedes edema formation and hypotension during sepsis. The angiogenic factor angiopoietin-1 (Ang-1) has vessel stabilizing effects, improving blood pressure and reducing mortality during lipopolysaccharide (LPS) exposure in mice [Witzenbichler et al. *Circ* 2005; 111 97-105]. The current study investigated whether a novel Ang-1 variant with improved solubility and activity (MAT.Ang-1), prevented loss of microvascular endothelial integrity in a murine model of sepsis [Cho et al. *Proc Nat Acad Sci* 2004; *101*, 5553-58]. The dorsal skinfold chamber was chronically implanted into male C3H/HEN mice (*n*=12, 7-10 weeks) to visualize fourth order striated muscle venules (<25microm.) using intravital microscopy. Mice received ketamine/atropine/xylazine (60/6/6mg.kg^{-1} i.p.) sedation during 0-4 and 20-24hrs post-LPS administration (1mg.kg^{-1} LPS at 0hrs and 19hrs), with and without MAT.Ang-1 (1.2mg.kg^{-1}, i.v. at 19 hrs). Fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA, 200µl.100g^{-1}, 20hrs i.v.) quantified macromolecular leak using computerized image analysis (IPPROPLUS). LPS induced leak (mean ±SEM) (40.7% at 22 hrs ±2.3 and 64.0% at 24hrs ±2.0, *n*=6), which was reduced by co-administration of MAT.Ang-1 (29.9% ±3.1 and 46.1% ±1.7 respectively, p=<0.05, *n*=6). Hence, MAT.Ang-1 appears to reduce microvascular endothelial damage during sepsis, which may contribute to maintenance of blood pressure and organ perfusion. Supported by British Heart Foundation Project grant and the Anaesthetic Research Society

KNL1

PULSED ACOUSTIC CELLULAR EXPRESSION (PACE) TREATMENT MODULATES LEUKOCYTE-ENDOTHELIAL INTERACTIONS IN MUSCLE MICROCIRCULATION

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We assessed in vivo the effect of Pulsed Acoustic Cellular Expression (PACE) on muscle microcirculatory hemodynamics, neovascularization, and leukocyte-endothelial interactions.
Rat cremaster model was used to evaluate microcirculatory hemodynamics of RBC velocity, capillary perfusion and leukocytes interactions over 4 hours. Rats were randomized into 5 groups: 1) Baseline controls (n=10); 2) PACE treatment: 15min before surgery (200 impulses) (n=8), 3) 500 impulses (n=8); 4) PACE treatment 24h before surgery (200 impulses) (n=8) and 5) 500 impulses (n=8). Immunohistochemistry assessed expression of cell adhesion molecules: E-selectin, CD15s, ICAM-1, VCAM-1 and proangiogenic factors: VEGF and vWF. PACE treatment increase the arteriolar diameters in groups 2 and 3 ($P<0.05$). Decrease in rolling leukocytes in groups 2 to 4, an increase in leukocyte adherence in groups 3 to 5, and increase in transmigrating leukocytes in group 4, compared to controls ($P<0.05$). Activation of adherent leukocytes correlated with upregulation of ELAM-1, their ligand CD15s and VCAM-1. Synergistic expression of VEGF and vWF was observed in all groups with upregulation of VEGF and vWF expression 24h after 500 impulses treatment. Application of PACE as preoperative treatment improves leukocytes-endothelial interactions and expression of proangiogenic VEGF and vWF factors.

Wednesday, August 27th
Leukocyte recruitment in inflammatory disease: role of endothelial chemokine binding sites
Parallel Symposium – S9

S9A

LEUKOCYTE RECRUITMENT IN INFLAMMATORY DISEASE: ROLE OF CHEMOKINE BINDING SITES

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Chemokines function in the trafficking of leukocytes from the blood into inflamed tissues. In order for chemokines to do this, they need to be localised at the luminal surface of endothelial cells. It is here that chemokines interact with chemokine receptors on the leukocyte cell surface resulting in integrin activation and transendothelial migration. Several receptors and other binding molecules have been implicated in immobilising chemokines at the endothelial surface, in addition to transporting these mediators across endothelial cells. These include the 'silent' chemokine receptor (or interceptor) Duffy antigen/receptor for chemokines (DARC) and carbohydrates such as glycosaminoglycans (GAGs). Other chemokine binding sites exist on endothelial cells, including signalling chemokine receptors (CXCRs and CCRs). In the synovium of rheumatoid arthritis patients, DARC, GAGs and chemokine receptors all occur in endothelial cells. The individual and collective functions of these binding sites are beginning to be revealed, and it will be interesting to understand how their functions resemble or differ from each other. Such knowledge would be useful in understanding central mechanisms in chronic inflammatory diseases.

[Supported by grants from Medical Research Council, BBSRC, Wellcome Trust, Astrazeneca, and Arthritis Research Campaign, all UK]

S9B

OVERCOMING THE BBB: MECHANISMS REGULATING THE CCL2:CCR2 AXIS DURING NEUROINFLAMMATION

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The chemokine CCL2 features critically in variety of neuroinflammatory and neurodegeneratrive diseases. As the major site of CCL2 production in the central nervous system is the astrocyte population, it has been challenging to resolve how this chemokine, secreted behind the highly impermeable bloods-brain barrier (BBB), can engage blood-born leukocytes. First, this laboratory evidenced the expression of CCL2 binding sites bearing pharmacodynamic properties of the CCL2 cognate receptor, CCR2, on the abluminal surface of isolated brain microvessels, Then, using an *in vitro* model of the BBB derived from brain microvascular endothelial cells (BMEC), this laboratory demonstrated that CCL2 can bind to the basolateral/abluminal BMEC surface and cause diminished expression and altered subcellular localization of tight junction (TJ)-associated proteins ZO-1 and occludin through a caveolin-1-dependent process. We further showed ^{125}I-CCL2 could be transported across BMEC in the basolateral/abluminal-to-apical/luminal direction, and that a component of this transport was sensitive to temperature, competition by unlabeled CCL2, knockdown of caveolin-1/caveolae and elimination of CCR2. In addition, immune-electron microscopy revealed that transported CCL2 co-localized with caveolin-1 in what appeared like vesicular structures that could be caveolae. Cell-conditional knockout mice have recently been developed to further probe the disposition of CCL2 *in vivo* during neuroinflammation.

This work was supported, in part, by grants 1RO-1-MH54718 and R21-NS057241 from the NIH, and grants RG-2633 and PP-1215 from the National Multiple Sclerosis Society to J.S. Pachter.

S9C

LEUKOCYTE EXTRAVASATION: AN IMMUNOREGULATORY ROLE FOR -L FUCOSIDASE?

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Fucosylated oligosaccharides and glycoconjugates have been implicated in several biological events, including the cell-cell adhesion processes, which mediate inflammation. Alpha-L-fucosidase (ALF) is an exoglycosidase, which is involved in the hydrolytic degradation of -L-fucose from glycoconjugates. In this study, we investigated the potential role of ALF in regulation of leukocyte migration. Measurement of trans-endothelial migration in response to CCL5 demonstrated that pre-treatment of monocytic cells with ALF reduced migration ($p=0.0004$) to a greater extent than treatment of the endothelial monolayer ($p=0.0374$). A murine model of experimental autoimmune uveitis was then used to show that treatment of splenic cells with ALF produced an 8.6-fold decrease in rolling and a 3.2-fold decrease in cell migration across the retinal vasculature. Further *in vitro* studies demonstrated that treatment of monocytes with the chemokines CCL3 or CCL5 increased the level of mRNA encoding ALF; this was accompanied by the detection of significant increases in protein. Treatment of monocytic cells with ALF for 2h significantly reduced the cell-surface expression of CD31, with a further

decrease in expression observed after 5h (p=0.002). Thus, CD31 seems to be one of the candidates through which ALF mediates its effect *in vitro*. These data identify a previously unrecognized immunoregulatory role for ALF in late stages of inflammation. This work was supported by grants from Northern Counties Kidney Research Fund and the Wellcome Trust

S9D
GLYCOSAMINOGLYCANS AS NOVEL ANTI-INFLAMMATORY MOLECULES
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Chemokines play an important role in the activation and direction of leukocytes during inflammation. They have two essential interactions: the first is a high affinity interaction with seven-transmembrane G-protein-coupled receptors (GPCRs) expressed on the surface of leukocytes, through which they exert cellular activation. The second is a low affinity interaction with extracellularly bound glycosaminoglycans (GAGs), enabling their immobilization to form a directional signal, through which leukocytes are directed towards the sites of inflammation. While cell-surface GAGs are essential for the *in vivo* activity of chemokines, soluble GAGs are able to inhibit their action. High affinity GAG fragments, that bind one or several chemokines, are therefore potential therapeutics for inflammatory and autoimmune diseases as well as cancer.
We have shown that heparin-based tetrasaccharides, which are a mixture of structures, are able to inhibit RANTES/CCL5 activity *in vivo*. We have tested homogeneous entities of the pool for their affinity towards RANTES and identified a tetrasaccharide with 20-fold increased affinity compared to the pool. We have also tested engineered heparins and related sugars and were able to identify a structure with a 100-fold increased affinity compared to low molecular weight heparin. However their activity *in vivo* is perplexing and will be discussed.

S9E
ON THE DARC SIDE
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Chemokines signal through their cognate G protein-coupled receptors to induce leukocyte migration and other cellular responses. Additionally, chemokines interact with interceptors, atypical “silent” heptahelical receptors which do not couple to G-proteins. These bind chemokines with high affinity but fail to transmit measurable intracellular signals in response. Duffy antigen/ receptor for chemokines (DARC) is an interceptor for almost all inflammatory chemokines. Besides erythrocytes, DARC is very prominently expressed on venular endothelial cells where its function has remained contentious. We show that DARC-transfected nuclear cells bind and efficiently internalize chemokines. However, in contrast to other known “silent” chemokine receptors, DARC does not target chemokines into the lysosomes for their intracellular degradation. Conversely, in polar cells grown on transwells DARC mediates unidirectional transport of chemokines from the baso-lateral to the apical cell surface where chemokines are retained intact and fully functional. Accordingly, the expression of DARC significantly increases the chemokine-induced *in vitro* leukocyte migration across the cellular monolayers. Furthermore, DARC transgenic mice, which overexpress this interceptor on the endothelial cells, show enhanced, whereas DARC deficient mice, diminished leukocyte extravasation into the sites of chemokine injections. These findings establish DARC as the chemokine transcytosis receptor on endothelial cells required for optimal chemokine- induced leukocyte emigration.

Wednesday, August 27th
Exercise-induced vascular adaptations I.
Parallel Symposium – S10

S10A
EXERCISE HYPEREMIA IN THE HEART
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Coronary blood flow is tightly coupled to myocardial oxygen consumption to maintain a consistently high level of myocardial oxygen extraction over a wide range of physical activity. This coupling depends on periarteriolar oxygen tension, signals from cardiomyocytes (adenosine, H_2O_2), the endothelium (prostanoids, nitric oxide, endothelin) and neurohumoral influences (catecholamines, angiotensin). These signals converge in signal transduction pathways that either alter opening of K-channels (K^+_{ATP}, $K+_{Ca}$, K^+_V), thereby altering membrane potential and intracellular Ca-concentration and/or affect Ca-sensitivity.
Yet, the individual contributions of these regulatory pathways, and their interactions, to exercise hyperemia in the coronary circulation are still incompletely understood. Studies in dogs, swine and humans have shown significant interspecies differences in control of coronary vasomotor tone. The coronary resistance vessels in swine lack significant alpha-adrenergic control, but are subject to beta-adrenergic feed-forward control during exercise. Moreover, nitric oxide, adenosine and K^+_{ATP}-channels contribute to resting coronary resistance vessel tone control in a linear additive manner, but are not mandatory for exercise hyperemia in the porcine heart. Rather, exercise hyperemia in swine involves K^+_{Ca}-channel-opening that is mediated, at least in part, by exercise-induced beta-adrenergic activation, possibly in conjunction with exercise-induced blunting of an endothelin-mediated vasoconstrictor influence. Supported by the Netherlands Heart Foundation (NHS2000T042).

S10B
ADAPTATION IN THE CONTROL OF SKELETAL MUSCLE BLOOD FLOW BY PHYSICAL ACTIVITY.
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Integration of current evidence indicates that training-induced vascular adaptation alters the determinants of skeletal muscle vascular resistance by at least two primary mechanisms: 1) Altered vasomotor reactivity of arteries and arterioles and 2) Structural remodeling of the arterial tree. A number of studies demonstrate that exercise hyperemia in skeletal muscle is altered by exercise training, and that changes in control of vascular resistance play a limited role in increased blood flow capacity in skeletal muscle. Evidence that physical activity induces both changes in vascular control and structure in skeletal muscle will be summarized in this paper. We believed that it was reasonable to propose that muscle fiber-type interacts with muscle fiber recruitment patterns during exercise so that the distributions of adaptive changes induced by exercise training vary with the type of training preformed. For example, high intensity exercise (high speed, uphill running) has been shown to produce the greatest relative increase in contractile activity in fast-twitch, white skeletal muscle, like the white portion of the gastrocnemius muscle. Following interval sprint training (IST), white gastrocnemius muscle exhibits the largest relative increase in oxidative capacity, capillary density, and blood flow capacity. In contrast, endurance exercise training (EX) has been shown to produce the greatest relative increase in contractile activity in the red portion of the gastrocnemius muscle (Gr). Importantly, Gr muscle also exhibits the largest relative increase in oxidative capacity and blood flow capacity of the extensor muscles examined in rats following EX training and capillarity increases in the Gr in EX rats. In contrast, capillarity is increased only in the white portion of the gastrocnemius muscle in IST rats. The limited magnitude of these changes indicate that increases in blood flow capacity of red and white gastrocnemius muscle following EX and IST training are not mediated solely by increased capillarity. Indeed, evidence indicates that changes in arteriolar density (number of arterioles/mm^2) contributes to regional increases in blood flow capacity within the gastrocnemius muscle and that exercise training induces increases in arteriolar density in muscle tissue with the greatest relative increase in fiber activity during training bouts. Computational hemodynamic network model analysis of vascular casts indicate that the primary adaptation to IST is the formation of new arterioles which, in turn, creates an approximate doubling of flow capacity and a deeper penetration of high pressure into the arteriolar network. (Supported by NIH Grant HL36088)

Wednesday, August 27th
Role of ageing and estrogen in the regulation of microvascular resistance
Parallel Symposium – S11

S11A
REGULATION OF FUNCTIONAL MUSCLE BLOOD FLOW IN OBESITY
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Obesity has been shown to impair functional vasodilation and thus blood flow in response to exercise. In obesity there is an impaired endothelium-dependent function due to alterations in nitric oxide, prostacyclin and thromboxane, leading to alterations in blood flow. Prostacyclin has been shown to be important in functional vasodilation during exercise. We propose that the impaired functional vasodilation in the obese Zucker rat (OZR), a model of obesity, is due to an imbalance between the vasodilator prostacyclin (PGI_2) and the vasoconstrictors thromboxane and its precursor, prostaglandin H_2. Inhibition of the thromboxane receptor (TP) improves functional vasodilation in the OZR as compared to the lean littermate, suggesting an increased vasoconstrictor response. In vitro experiments demonstrate that PGI_2 release is impaired from isolated arteries, which may be due to the observed nitration of PGI_2 synthase. PGI_2 causes dilation though activation of K_{ATP} channels which are also inhibited in obesity. Rosiglitazone (PPAR activator) treatment, which improves insulin resistance and oxidative stress, increases functional vasodilation via diminished TP activation and increased K_{ATP} channel function in OZR. Together these results suggest that insulin resistance and the resultant increase in ROS in obesity can have inhibitory effects on vascular responses to exercise. Supported by NIH HL-51971 and HL-63958

S11B
CONTRIBUTION OF INSULIN RESISTANCE TO VASCULAR DEFECTS IN OBESITY
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Obesity is the most serious risk factor for cardiovascular disease but the links between obesity and specific cardiovascular dysfunctions are incompletely understood. Using mouse models of obesity, we have described a number of cardiovascular dysfunctions, including impaired endothelium-dependent vasodilation, increased sympathetic activation of blood pressure and inward microvascular remodeling. To determine the role of insulin resistance (IR), protein tyrosine phosphatase 1B (PTP1B), a termination pathway for insulin signaling, was deleted on the *db/db* background to produce obese mice in which IR was reversed. These dual knockout *db/db* PTP1B null mice remained morbidly obese but had corrected markers of peripheral IR: improved glucose tolerance, reduced HbA1C and normalization of dyslipidemia. Surprisingly, markers of hepatic IR such as fasting glucose or hyperinsulinemia persisted, suggesting that deletion of PTP1B in *db/db* mice specifically corrects peripheral IR. Compared to normal *db/db* mice, PTP1B-null *db/db* mice showed normalization of endothelial function and improved microvascular remodeling. Sympathoactivation of arterial pressure persisted despite deletion of PTP1B, indicating that other aspects of obesity account for this deficit. Taken together, these data indicate that specific relationships exist in obese individuals between risk factors and vascular dysfunctions and understanding these relationships will further our understand of cardiovascular disease in obesity.

S11C
ENDOTHELIAL GLYCOCALYX PROPERTIES IN OBESITY
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The glycocalyx is a gel-like layer of ~0.5 – 1.0 µm which emerges as an important protective interface between the endothelium and circulating blood. Using tracer dilution of glycocalyx-selective tracers, we recently assessed glycocalyx properties in the systemic circulation in obese (and hyperglycemic, db/db) mice. In db/db mice of 6-8 weeks old, exclusion of circulating blood was not affected compared to control C57Bl/6 mice, yet exclusion of FITC-labeled 70 kDa dextrans, having limited access to the glycocalyx in control mice, was impaired in the db/db mice. Further, robust systemic leakage of the dextrans was observed in the db/db mice. Treatment of the db/db mice with metformin for 2 weeks resulted in an improvement of glycocalyx exclusion and barrier properties, without an effect on fasting blood glucose levels. In addition, metformin treatment partly restored dehydration of heart and kidney in the db/db mice. In conclusion, conditions of obesity and hyperglycemia are associated with compromised glycocalyx properties, but these can be partly reversed by metformin treatment. We envision that early perturbation of glycocalyx contributes to obesity-related microvascular dysfunction. Supported by Dutch Diabetes Research Foundation grant 2006.00.027 and Netherlands Heart Foundation grant 2005T073

S11D
INFLUENCE OF HIGH-FAT DIET ON VASODYNAMIC PARAMETERS IN DIFFERENT MOUSE STRAINS
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In this study, we tried to circumvent the limitations of standard organ chamber experiments using in vivo optical coherence tomography (OCT) to analyze the vascular function of small arteries of different mouse strains. OCT images were acquired with a two-axis galvanometer scanner head. Time series (4 frames per second) of cross-sectional images were analysed with image processing software measuring the time course of the vessel lumen dynamics. Vascular function of murine saphenous artery and vein of male C57BL/6 (WT) and LDLR knockout (LDLR k.o.) mice was first analyzed at 6 weeks of age (WT n=32; LDLR k.o. n=13). The second measurements were performed at 20 weeks of age after a 14 week feeding period of control (14 weeks control diet; WT n=15; LDLR k.o. n=8) or high-fat diet (14 weeks high-fat diet; WT n=18; LDLR k.o. n=7) containing 21.2% butter fat and 2.1 mg/kg cholesterol. Vasoconstriction was analyzed in response to 80 mM K^+, vasodilatation in response to 1 mM SNP. The results of our study are summarized in the table (mean SEM).

	C57BL/6			LDLR knockout		
	6 weeks	14 weeks control diet	14 weeks high-fat diet	6 weeks	14 weeks control diet	14 weeks high-fat diet
diameter in µm						
resting condition	122±6.4	172±8.9	171±6.5	119±9.2	150±9.6	146±4.8
vasoconstriction	9.6±3.0	37.9±6.6	44.8±4.8	5.7±4.4	12.1±6.1	21.9±8.3
vasodilatation	227.0±4.0	245.0±7.6	238.0±7.6	196±10.0	220±9.6	238±4.9
Vmax in µm/s						
vasoconstriction	16.1±2.0	14.6±1.4	12.1±0.8	15.2±1.7	17.8±2.4	17.4±1.4
vasodilatation	20.2±2.6	19.4±1.6	19.6±2.7	14.3±2.2	16.2±3.3	16.8±2.3
t(1/2) in s						
vasoconstriction	4.5±0.4	6.5±0.4	8.3±1.0	7.4±1.0	5.3±1.0	9.0±1.6
vasodilatation	8.5±1.2	9.8±0.5	11.8±0.6	10.3±1.9	11.1±1.2	12.0±0.9

In conclusion, using OCT technique we were able to image the vascular function of small arteries of different mouse strains in response to high-fat diet in vivo. This imaging procedure offers the ability to display differences in early stages of atherosclerotic pathogenesis.

S11E

MUSCLE MICROVASCULAR FILTRATION CAPACITY AND BLOOD FLOW IN METABOLIC SYNDROME

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Metabolic syndrome is a combination of risk factors that increase risk of cardiovascular disease (CVD) and diabetes. We have assessed muscle microvascular function in 38 consented volunteers [52+/-9y, n=17 male] with two or more features of metabolic syndrome (central obesity, dyslipidaemia, high blood pressure and dysglycaemia), before and after 6 months lipid lowering therapy (Atorvastatin 40mg o.d.) or placebo control. Filtration capacity (Kf), isovolumetric pressure (Pvi) and resting limb blood flow (Qa) were measured using strain gauge plethysmography (FiltrassAngio®, Compumedics.dwl, Germany). At baseline, Kf was negatively correlated with overall CVD risk, visceral fat, insulin resistance, LDL Cholesterol, HbA1c,markers of inflammation and age ($r>0.4$, $p<0.05$), and positively correlated with HDL cholesterol. Qa was related to activity ($r=0.42$, $p=0.03$). These individuals also showed a marked decrease in dilator capacity. While statins lowered LDL cholesterol ($P<0.0001$), they had little effect on any of our microvascular measures. These data suggest an attenuation in capacity for blood/tissue exchange in people with features of metabolic syndrome. Although statins are known to decrease risk of CVD, we were unable to show any improvements in microvascular function over the 6 months study.

S11F

MALFUNCTION OR ADAPTATION IN THE MICROCIRCULATION-CONTROVERSIES IN OBESITY

Z Bagi

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Obesity is believed to impair the function of large coronary vessels. However, the impact of obesity on the function of coronary resistance vessels, known to determine tissue perfusion of the heart, is still debated. Interestingly, recent studies propose the existence of several adaptive mechanisms that may maintain the dilatory function of coronary microvessels during the disease development. Among others, reactive oxygen species (ROS) and activation of vascular inflammatory pathways have been implicated to compensate for the loss of vasodilator function. Particularly, hydrogen peroxide (stable form of ROS) and cyclooxygenase-2-derived dilator prostaglandins has been shown to enhance the dilatory function of coronary microvessels. Importantly, clinical studies demonstrated that a potential interference with these adaptive signaling

mechanisms, such as by the use prostaglandin synthesis inhibitors and antioxidants would paradoxically temper the dilator function of coronary microvessels, predisposing these patients to tissue ischemia. A better understanding of adaptive mechanisms in coronary microvessels may gain insight into the complex chain of pathological events in obesity, and may explain some of the existing controversies in the therapeutic outcomes.

Support: Hungarian National Scientific Research Fund (OTKA) F-048837

Wednesday, August 27th
Intercellular signaling in the control of vascular resistance
Parallel Symposium – S12

S12A
DISSECTING THE ROLE OF CONNEXIN ISOFORMS IN CONDUCTED VASODILATION
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Intercellular communication ensures a coordinated behaviour of vascular cells, which is reflected by uniform diameter changes of arterioles over considerable distances. Conducted dilations can be elicited by local application of acetylcholine (ACh), bradykinin or adenosine. These substances hyperpolarize the cells, which are thought to be the signal that elicits a coordinated dilation. The locally initiated hyperpolarization spreads along the vessel through intercellular channels (gap junctions) composed of connexins (Cx). In vascular cells Cx40, Cx43, Cx37, and Cx45 are expressed, of which Cx40 is especially important because conducted dilations initiated by ACh or bradykinin are attenuated in Cx40-deficient animals. The function of Cx40 cannot be replaced by Cx45 expressed instead of Cx40, which suggests that specific Cx-properties are necessary to support conduction along the endothelium in which Cx40 is abundantly expressed. In contrast to ACh and bradykinin, conducted dilations initiated by adenosine are not attenuated in Cx40-deficient mice suggesting a distinct pathway possibly the smooth muscle cell layer. This is further supported by divergent properties of conducted dilations initiated by adenosine or ACh. Our data thus indicate that distinct pathways composed of different connexins are provided in arterioles to promote a coordinated diameter changes along the vessel length.

S12B
MYOENDOTHELIAL COUPLING AND THE COORDINATON OF SMOOTH MUSCLE RELAXATION
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The projection of endothelial cells through holes in the internal elastic lamina to make close contact with smooth muscle cells forms a unique signalling microdomain. The close apposition to smooth muscle cells makes this an ideal site for the release of diffusible factors from endothelial cells, in addition to direct cell-cell coupling. In rat mesenteric arteries, the rises in intracellular Ca^{2+} within this domain can activate $K_{Ca}2.3$ and $K_{Ca}3.1$ channels which, together with the Na^+/K^+-ATPase, are expressed in this microdomain. However, unlike $K_{Ca}2.3$, $K_{Ca}3.1$ channels are dynamically regulated by both extracellular Ca^{2+} levels and PKA. This provides an opportunity to modulate opening of these K^+ channels depending on the active state of smooth muscle cells, and provides a pathway for signalling to smooth muscle not dependent on myoendothelial gap junctions. In contrast, $K_{Ca}2.3$, but not $K_{Ca}3.1$ channels are also strongly expressed at the borders between endothelial cells, and heterocellular electrical coupling is the predominant mechanism for evoking smooth muscle cell hyperpolarization and relaxation. Overall, these data suggest that K^+ and myoendothelial coupling evoke endothelium-dependent hyperpolarization-associated relaxation through distinct, definable pathways.

S12C
ROLE OF Cx37 AND Cx40 IN INTER-ENDOTHELIAL ELECTRICAL COUPLING DURING INFLAMMATION
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We examined the effect of lipopolysaccharide (LPS, an initiating factor in sepsis) on electrical coupling *in vitro*, in monolayers of microvascular endothelial cells (male mouse hindlimb muscle origin). To assess coupling, we measured the spread of electrical current injected into the monolayer. LPS reduced coupling in wild type (WT), $Cx37^{-/-}$, $Cx43^{G60S}$ (non-functional mutant), but not in $Cx40^{-/-}$ cells. In WT cells, the reduction was tyrosine kinase-, ERK1/2-, PKA-, and PKC-dependent, mediated via PKA-specific serine dephosphorylation of Cx40. Significantly, LPS and hypoxia/reoxygenation (confounding factor in sepsis) synergistically reduced coupling and enhanced Cx40 dephosphorylation. We also examined the effect of NO (a factor in advanced sepsis) on coupling. Sepsis reduced arteriolar conducted vasoconstriction in WT, $iNOS^{-/-}$, $eNOS^{-/-}$, but not in $nNOS^{-/-}$ mouse cremaster muscle *in vivo*, cGMP-independently. In monolayers *in vitro*, exogenous NO rapidly reduced coupling in WT, $Cx40^{-/-}$, $Cx43^{G60S}$, but not in $Cx37^{-/-}$ cells, also cGMP-independently. In WT cells, NO scavenger, but not superoxide or peroxynitrite scavenger, eliminated this reduction. We propose that sepsis initially reduces electrical coupling along the microvasculature via PKA-specific serine dephosphorylation of Cx40. Hypoxia/reoxygenation enhances this effect. During later stages of sepsis associated with nNOS-derived NO overproduction, NO closes gap junctions by targeting Cx37, cGMP- and oxidant-independently. (Support: CIHR and HSFO).

S12D

INTERACTION OF SHEAR STRESS AND MEMBRANE POTENTIAL IN REMOTE VASODILATIONS

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Complex vasoactive responses are elicited by remote application of ligands. We concentrate on responses observed within arteriolar networks. Ligation of the □v□3 integrin receptor downstream with LM609 or VEGF (not bFGF) induces a cascade beginning with an immediate increase in velocity prior to any change in diameter. Seconds later, upstream regions dilate in a biphasic manner. The net response increases flow into the network by 100%; that flow passes to the downstream arteriolar branch that was stimulated. One component of the dilation is blocked with LNNA at the upstream location, suggesting that the elevated flow causes flow mediated dilation. A second component of the dilation is blocked when gap junctional signaling along the network is blocked. We used a pharmacological approach to investigate the role of potassium (causing local hyperpolarization) and chloride (causing local depolarization) channels that are activated by ligation of the □v□3 integrin receptor. Preventing depolarization downstream blocked the initial increase in velocity, and part of the dilation. Preventing hyperpolarization downstream did not prevent the velocity increase, and instead required gap junctions. Together, this response provides a redundant mechanism to elevate flow into an arteriolar network, which directs that flow to the stimulated site. Supported by: NIH, AHA.

S12E

INTEGRATING ELECTRICAL AND CALCIUM SIGNALING IN RESISTANCE NETWORKS

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Increasing tissue blood flow requires synchronous relaxation of smooth muscle cells (SMC) to produce vasodilatation along resistance networks. We have investigated roles for electrical and calcium signaling in coordinating dilatation of microvessel branches. In isolated pressurized feed arteries of hamster retractor muscle with spontaneous resting tone (diameter, 50-70 microns; membrane potential, -30 to -40 mV), ACh microiontophoresis triggers a local increase in endothelial cell $[Ca^{2+}]_i$ to activate KCa channels and produce hyperpolarization (10-15 mV). Hyperpolarization conducts rapidly (cm/s) and bidirectionally along endothelium and into consecutive SMC through homocellular and myoendothelial gap junctions, closing L-type Ca^{2+} channels to produce electromechanical relaxation and vasodilatation. An ensuing 'wave' of $[Ca^{2+}]_i$ (Fluo-4 confocal imaging) also travels bidirectionally along endothelium (~ 0.1 mm/s), releasing NO to sustain vasodilatation through pharmacomechanical relaxation of successive SMC. Inhibiting KCa channels (apamin + charybdotoxin) eliminates rapid conduction but not endothelial calcium waves or pharmacomechanical vasodilatation. In cremaster muscle of transgenic mice expressing a $[Ca^{2+}]_i$ sensor (GCaMP2) in arteriolar endothelium, ACh microiontophoresis initiates conducted vasodilatation without $[Ca^{2+}]_i$ responses at remote sites, followed by secondary dilatation upon arrival of a calcium wave. These findings illustrate complementary electrical and calcium signaling pathways along microvascular endothelium for coordinating vasodilatation in resistance networks. (Support: NIH HL41026 & HL56786; MI Kotlikoff & NY Tallini @ Cornell University)

S13A
AXIAL AND CIRCUMFERENTIAL UTERINE ARTERIAL REMODELING IN NORMOTENSIVE VS. HYPERTENSIVE PREGNANCY
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Using an established model of hypertension (NO inhibition with L-NAME) in nonpregnant and pregnant rats, comparisons were made to age-matched nonpregnant and pregnant controls; others received hydralazine (25 mg/kg/day) along with L-NAME during pregnancy to prevent blood pressure elevation while maintaining NO inhibition. Arterial length, lumen diameter and wall thickness were measured late in pregnancy (day 20/22) in isolated, pressurized segments of the main uterine artery, and in smaller radial arteries. Pregnancy resulted in doubling of main uterine artery length, and a three-fold increase in radial artery length; elongation was completely unaffected by hypertension/NO inhibition. Expansive (circumferential) remodeling of the main uterine artery was inhibited by L-NAME treatment; co-administration of hydralazine was without effect. Smaller radial arteries also underwent widening and elongation during gestation, and although circumferential remodeling of these vessels was attenuated in hypertensive animals, significant improvement was observed in those co-treated with hydralazine. These data underscore the complexity of gestational vascular remodeling in terms of vessel size (large vs. small), pattern (axial vs. circumferential), and cause (NO inhibition vs. hypertension). Interestingly, neither hypertension nor NO inhibition had any measurable effect on arterial elongation, a process that occurs through mechanisms that have yet to be identified. Supported by NIH RO1 HL 073895

S13B
EFFECTS OF ESTROGEN ON MITOCHONDRIAL FUNCTION IN THE CEREBRAL CIRCULATION.
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In vivo treatment with 17beta-estradiol (E) enhances efficiency of energy production in cerebral blood vessels, while suppressing mitochondrial oxidative stress. Therefore cultured human brain microvascular endothelial cells (HBMEC) were treated with 10 nM E for 24 hr to investigate mechanisms of this effect. E increased mitochondrial cytochrome c protein and mRNA; use of silencing RNA for estrogen receptors (ER) demonstrated ERalpha, but not ERbeta involvement. Activity of aconitase, containing an iron-sulfur center inactivated by mitochondrial superoxide, was measured to indicate mitochondrial ROS. E increased mitochondrial aconitase activity, indicating reduced ROS. Direct measurement of mitochondrial superoxide with MitoSOX Red showed that E, but not 17alpha-estradiol, decreased mitochondrial superoxide production, an effect blocked by an ER antagonist. Selective ER agonists demonstrated that the decrease in mitochondrial superoxide was mediated by ERalpha, not ERbeta. E did not affect Mn superoxide dismutase mRNA, protein or activity. Instead, ERalpha–mediated decreases in mitochondrial ROS may depend on the concomitant increase in mitochondrial cytochrome c, previously shown to act as an antioxidant. Effect of E to decrease ROS production in the cerebral endothelium may contribute to sex differences in the pathophysiology of stroke and other age-related neurodegenerative diseases.

S13C
ADAPTATION FOR NO DEFICIENCY; ROLES OF ESTROGEN AND EDHF
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By using EDHF bioassay, we tested the hypothesis that female arteriolar endothelium releases a vasoactive substance in response to shear stress, to hyperpolarizes smooth muscle cells. Increased shear stress elicited by increases in perfusate flow, were applied to the donor vessel and changes in arteriolar diameter and smooth muscle membrane potential of the detector vessel were recorded. The hyperpolarization/vasodilation were abolished by iberiotoxin. Concentrations of EETs in the perfusate collected from shear stress-stimulated female NO deficient donor vessels dose-dependently increased. Thus, EETs released from endothelium of the donor vessel hyperpolarize/dilate the detector vessel, via activation of BK channels of smooth muscle. We used microarray analysis to screen the global gene of cytochrome P450 (CYP) in NO deficient microvessels and demonstrated that CYP2C 29 gene expression in microvessels was significantly increased in female eNOS-KO mice. This upregulation of CYP2C 29 was associated with an upregulation of transcription factor RXR . Transfection of the vessels with specific CYP2C 29 or RXR gene siRNA significantly inhibited EET-mediated flow-induced dilation, indicating that these specific genes play roles in the signal transduction pathway, via activation of RXR by estrogen, which in turn, upregulates downstream CYP2C 29 gene to increase EET synthesis. (Supported by NIH HL 070653, HL 68813, and HL 43023)

S14A
DYNAMIC IMAGING OF CANCER CELLS IN THE MICROCIRCULATION
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Imaging of fluorescent-protein-labeled cells has led to the new science of in vivo cell biology (Nature Reviews Cancer 5, 796-806, 2005). With the use of cancer cells labeled with green fluorescent protein (GFP) in the nucleus and red fluorescent protein (RFP) in the cytoplasm and a highly sensitive imaging system with both macro-optics and micro-optics, we have developed subcellular real-time imaging of cancer cell trafficking in the microcirculation of live mice. We could observe cytoplasmic and nuclear dynamics of tumor cells trafficking in blood vessels in live mice since they were differentially labeled with GFP and RFP. During extravasation, real-time dual-color imaging showed that cytoplasmic processes of the cancer cells exited the vessels first, with nuclei following along the cytoplasmic projections. Both cytoplasm and nuclei underwent deformation during extravasation. Different cancer cell lines seemed to strongly vary in their ability to extravasate. Using the same approach, we showed real-time imaging of dual-color cancer cell trafficking in lymphatic vessels. The double-labeled cancer cells trafficked through lymphatic vessels where they were imaged in real time. The subcellular dynamics of cancer metastasis can now be observed in live mice in real time, which will enable further understanding of the critical steps of metastasis and provide novel visible targets for antimetastasis drug development.

S14B
IMAGING AND MANIPULATION OF CELLS AND MOLECULES WITH ATOMIC FORCE MICROSCOPY AND FLUORESCENCE-AFM COMBINATIONS
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The atomic force microscope (AFM) is a high-resolution scanning-probe instrument, which has become an important tool for cellular and molecular biophysics in recent years. Although excellent in spatial resolution, it lacks the time resolution and functional specificities offered by fluorescence microscopic techniques. To exploit the advantages of both methods, we developed a spatially and temporally synchronized total internal reflection fluorescence and atomic force microscope system. The instrument, called STIRF-AFM, is a stage-scanning device in which the mechanical and optical axes are co-aligned to achieve spatial synchrony. At each point of scan, the sample topography (AFM) and fluorescence (photon count or intensity) information are simultaneously recorded. The tool was tested and validated on various cellular (monolayer cells in which actin filaments and intermediate filaments were fluorescently labeled) and biomolecular (actin and desmin filaments, titin molecules and fragments, amyloid fibrils) systems. We demonstrate that with the technique correlated sample topography and fluorescence images can be recorded, the surface of living cells can be mechanically manipulated in a targeted fashion, and the fluorescence of mechanically manipulated molecules can be followed with high temporal resolution.
Supported by grants from the Hungarian Science Foundation (OTKA T049591), Hungarian National Office for Research and Technology (KFKT-1-2006-0021) and the Hungarian Ministry of Health (ETT-506/2006).

S14C
TWO-PHOTON MICROSCOPY AND ITS APPLICATIONS IN (MICRO) VASCULAR RESEARCH
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Two-photon microscopy combines optical sectioning with a good depth of penetration. Excitation is performed using a pulsed laser at a wavelength in the order of 800nm. If two photons arrive simultaneously, they deliver the required energy normally supplied by one photon. The volume of excitation and, hence, the volume of emission and the resolution of the optics is determined by the excitation wavelength. Good resolution was obtained in large arteries up to a depth of 200microns. Using fluorochromes, such as quantum dots, specific structures were labeled. Quantum dots have high quantum yields, high photo-stability and a broad excitation/narrow emission spectrum, enabling long-term, multicolor imaging with a single wavelength excitation source. Studies have been performed *ex-vivo* in isolated blood vessels and *in-vivo*. Functionality in the *ex-vivo* experiments was tested: absence of leaky cells, good reaction to pharmacological stimuli, and comparison with wire-injury. Structures and alterations were studied in large arteries (elastic versus muscular; common carotid and bifurcation) during (early) atherosclerosis in $ApoE^{-/-}$ and in control mice: elastin layers, cell nuclei, collagen, apoptosis, endothelial glycocalyx, permeability of endothelium, atherogenesis, and angiogenesis of vaso vasorum. Furthermore, neovascularization in tumors was imaged. Data and 3D-reconstructions will be presented demonstrating the possibilities of the method.

S14D

MICRO-MYOGENIC EVENTS INDUCED IN VASCULAR SMOOTH MUSCLE BY MECHANICAL FORCE APPLIED TO A SINGLE FIBRONECTIN FOCAL ADHESION

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We investigated the responses of microvascular smooth muscle (mVSMC) to mechanical force applied through adhesion sites involving the extracellular matrix (ECM)-integrin-cytoskeletal (CSK) axis. Using an integrated atomic force microscope (AFM) – fluorescence optical microscopy system, forces were measured and applied with the AFM. Using AFM, adhesion force between integrins and ECM, mVSMC mechanical responses and cell mechanical properties were measured. AFM probes were conjugated with fibronectin (FN) or other ECM proteins and interacted with the mVSMC membrane. RGD-containing peptides and function blocking antibodies against a5 or b1 integrins blocked FN-mVSMC adhesions demonstrating the selectivity of the interaction with a5b1 integrin. AFM cantilevers with an FN coated 5μm diameter bead on the tip were used to establish individual focal adhesions composed of integrins and CSK proteins. Application of a pulling force to the bead-ECM cell contacts induced a micro myogenic event in which force was generated to oppose the applied force. By comparison, application of force to beads coated with collagen I, vitronectin, or laminin did not induce micro myogenic events. These studies support an important role for FN in mVSMC mechano-sensation and -transduction and demonstrate the utility of AFM for investigations of cellular responses to mechanical forces.

Wednesday, August 27th
Microvasculature in skeletal muscle
Parallel Symposium – S15

S15A
MORPHOLOGY OF THE MICROVASCULATURE IN SKELETAL MUSCLE
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Mammalian skeletal muscles are composed of a specific mosaic of muscle fibers surrounded by capillaries that provide the tissue with oxygen and nutrition. The relationship between skeletal muscle fibers and capillaries is dynamic. Changes in the microcirculatory ultrastructure in response to different stimuli (mechanical, hemodynamic, environmental) have been documented at the ultrastructural level in many cases. The tortuosity of the capillary network might vary in respect to fiber type and blood flow. Simultaneous increases in the capillary-to-fiber ratio and the capillary density indicate that the capillary system has expanded (a process-designated angiogenesis). The basement membrane and the recruitment rate of peri-endothelial cells can be distinctly enlarged as found in patients suffering from peripheral arterial disease and diabetes. The phenotype of endothelial cells depends on the availability and levels of oxygen in the tissue. Such examples for the plasticity of the microcirculation in skeletal muscle will be presented and discussed in terms of functional impact. Support: Swiss National Science Foundation

S15B
MORPHOLOGY OF THE MICROVASCULATURE IN SKELETAL MUSCLE
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In mature skeletal muscle, the main stimuli initiating capillary endothelial proliferation are hypoxic up-regulation of vascular endothelial growth factor (VEGF), haemodynamic shear stress, and inflammatory mediators, but growth of new capillaries and supplying arterioles depends on complex interactions when these conditions co-exist. In rat hindlimb ischaemia as a model of low flow, tissue hypoxia and VEGF up-regulation, small arterioles are lost and angiogenesis is restricted because of reduced capillary shear stress and impaired VEGF receptor 2 expression. These morphological changes can be mimicked by chronic inhibition of NO synthase, which impairs vasodilation without altering VEGF or receptor expression. This emphasizes the importance of blood flow as an angiogenic modulator in the microcirculation, *via* shear stress and NO. The rescue of small arterioles in ischemic muscle by L-arginine treatment, which restores vasodilation, also shows that flow is necessary for remodeling. Muscle activity remains the most effective means of inducing angiogenesis and arteriolar growth through a combination of transient tissue hypoxia, elevated capillary shear stress, VEGF and VEGF receptor up-regulation and mild inflammation. Applied to ischemic muscles, activity restores rescues both capillaries and arterioles. This approach has been used in human muscle, both healthy and ischemic, to induce microvascular growth.

S15C
METABOLIC SYNDROME AND MICROVASCULAR RAREFACTION: CONTRIBUTIONS FROM NITRIC OXIDE AND INFLAMMATION
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Obesity, with the combined presentation of additional CVD risk factors including dyslipidemia, insulin resistance and hypertension, represents a considerable public health challenge. When combined, these pathologies are defined as the metabolic syndrome, with a profound implication of this state being an increased risk for negative vascular outcomes. The obese Zucker rat (OZR) possesses a dysfunctional leptin receptor gene, causing hyperphagia-induced evolution of the metabolic syndrome and an array of negative vascular outcomes, including alterations to reactivity and microvascular density (MVD). Previous studies indicate that reductions in skeletal muscle MVD in OZR are best temporally predicted by insulin resistance severity, and are tightly associated with a progressive reduction in vascular nitric oxide (NO) bioavailability. Interventions against elements of the metabolic syndrome (pharmacological) or against the metabolic syndrome itself (exercise) improved MVD, dependent on increased NO bioavailability. Also identified were improvements to specific markers of inflammation associated with microvessel loss and reduced vascular NO bioavailability. Of these, the earliest to demonstrate a significant change from baseline in OZR is TNF-□, preceding alterations to either MVD or NO bioavailability. Chronic inhibition of TNF-□ (pentoxyfillene) improved vascular NO bioavailability and blunted the reduced MVD in OZR. Taken together, these results suggest that microvessel loss in the metabolic syndrome may represent an extremely complicated, multi-factorial process where patterns of peripheral inflammation integrate to reduce vascular NO bioavailability. It may be that this loss in NO bioavailability alters angiogenic/angiostatic growth factor profiles which result in microvessel loss. (NIH DK64668; AHA 0740129N)

S15D

NANOPARTICLE INHALATION ACTIVATES INFLAMMATORY MECHANISMS AND IMPAIRS SYSTEMIC MICROVASCULAR FUNCTION

TR Nurkiewicz [1], DW Porter [2], AF Hubbs [2], JL Cumpston, BT Chen [2], D Frazer [2], V Castranova [2], MA Boegehold [1]

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We have shown that pulmonary exposure to particulate matter impairs endothelium dependent dilation in systemic arterioles. The purpose of this study was three-fold: 1) determine if nanoparticles similarly impair microvascular function, 2) characterize alterations in endogenous nitric oxide (NO) production after nanoparticle exposure, and 3) identify systemic mechanisms that may influence endogenous NO production after nanoparticle inhalation. Rats were exposed to titanium nanoparticles via inhalation (primary particle diameter ~21 nm) at depositions of 4-90 g/rat. The spinotrapezius muscle was prepared for intravital microscopy 24 hrs after exposures. Intraluminal infusion of the Ca^{2+} ionophore A23187 was used to evaluate endothelium-dependent arteriolar dilation. Endogenous microvascular NO production was measured with an electrochemical NO sensor. Microvascular oxidative stress was quantified with dihydroethidium fluorescence. In control rats, A23187 produced dose-dependent arteriolar dilations. In nanoparticle exposed rats, A23187 produced arteriolar constrictions or significantly impaired vasodilator responses. Nanoparticle exposure increased microvascular oxidative stress by ~60%, and decreased NO production in a dose-dependent manner. Treatment with either 4-aminobenzoic hydrazide (myeloperoxidase inhibitor) or apocynin (NADPH oxidase inhibitor) partially restored NO production and normal microvascular function. These results are consistent with the hypothesis that nanoparticle exposure activates systemic inflammatory mechanisms that disturb normal microvascular function. Support: R01-ES015022 and HEI#4730 (TRN).

Wednesday, August 27th
Microvascular actions of insulin in muscle and underlying mechanisms
Parallel Symposium – S16

S16A

OVERVIEW OF INSULIN'S MICROVASCULAR ACTION AND INSULIN RESISTANCE USING BIOCHEMICAL REPORTER AND ULTRASOUND IMAGING TECHNOLOGIES

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Impaired insulin-mediated glucose uptake in skeletal muscle is a hallmark of insulin resistance associated with obesity, hypertension and diabetes. Insulin mediates its own access and that of glucose to muscle by regulating both bulk and microvascular blood flow. Several new technologies, which are based on methodologies such as a biochemical reporter for capillary blood flow, microdialysis recovery of tracers, Laser Doppler implantable probes, or contrast-enhanced ultrasound, allow measurement of microvascular blood flow in vivo. These techniques have demonstrated that microvascular flow can be regulated by insulin independently of bulk blood flow. Additionally the loss of insulin-mediated recruitment of microvascular blood flow is a characteristic of insulin resistance states. Factors that diminish insulin's action upon the microvascular blood flow lead to acute insulin resistance in terms of muscle glucose uptake. These factors include fatty acids, inflammatory cytokines, hypertensive agents such as endothelin, and physical occlusion of the microvasculature with latex microspheres. Together these data suggest a compelling connection between the loss of insulin-mediated microvascular action within skeletal muscle and the development of metabolic insulin resistance. The author is supported by grants from Nation Health & Medical Research Council of Australia, National Heart Foundation of Australia, and the National Institutes of Health, USA.

S16B

INTERSTITIAL SAMPLING AND INJECTION STUDIES TO ASSESS MICROVASCULAR RESPONSES AND INTERVENTIONS THAT RESULT IN INSULIN RESISTANCE

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In vivo investigations in interstitial fluid of insulin sensitive tissues have an important role in the investigation of tissue perfusion and local cellular fuel supply at the microvascular level. In particular, in relation to the development of diabetes and insulin resistance, the role of subcutaneous adipose tissue has been underestimated and recent investigations clearly indicate that the adipocyte also has an important endocrine function beyond its role as a fat storage depot. In this regard, the specific role of insulin to mediate fuel supply to insulin sensitive tissues at the microvascular level may be expanded and more generalized as the role of insulin as a regulator of the "cross-talk" between organs.

However, there is an ongoing debate about the role of insulin to mediate the microvascular response and also on the possible role of a defect of this insulin stimulated microvascular response as an additional factor contributing to the development of insulin resistance.

Recent investigations applying the lymph drainage technique in dogs are clearly in support of the role of insulin in the regulation of tissue perfusion and also demonstrated a relation between this effect and the development of insulin resistance. In contrast, a study performed in humans was not able to demonstrate this observed effect in human subcutaneous adipose tissue or skeletal muscle.

The present talk will highlight the most important studies related to this topic, and focus on the demonstration of the difficulties of methodological aspects related to the study of in vivo interstitial fluid metabolism in humans.

S16C

REGULATION OF INSULIN-MEDIATED VASOREACTIVITY IN MUSCLE BY ADIPOSE TISSUE

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Obesity is associated with insulin resistance and endothelial dysfunction, and endocrine products of adipose tissue (adipokines) have been implicated in the underlying mechanisms. We have studied the hypothesis that insulin's microvascular effects in muscle are directly regulated by the fatty acid palmitic acid, the cytokine tumor necrosis factor alpha (TNF) and adiponectin in an antagonistic fashion. In vasoreactivity studies in the pressure myograph on isolated resistance arteries from the rat cremaster and mouse gracilis muscle, we found that insulin enhances both nitric oxide (NO) and endothelin (ET-1) activity, resulting in a neutral effect on vascular diameter in the absence of adipokines. In the presence of palmitic acid or tumor necrosis factor alpha (TNF), insulin induced a dose-dependent vasoconstriction. This shift in insulin-mediated vasoreactivity was associated with a selective inhibition of insulin-mediated activation of Akt, which mediates insulin-induced activation of endothelial NO synthase. The interference of palmitic acid and TNF with insulin-mediated vasoregulation was critically dependent on activation of the kinases c-jun N-terminal kinase (JNK) and protein kinase C theta (PKCθ) in the vascular endothelium, which was studied by three-dimensional imaging of the vessel wall. In contrast to the

effects of palmitic acid and TNF , adiponectin shifted insulin-mediated vasoreactivity in muscle resistance arteries to dose-dependent vasodilatation, which was associated with selective inhibition of insulin-mediated activation of extracellular-regulated kinase 1/2 (ERK1/2), which mediates insulin-induced ET-1 release. The interference of adiponectin with insulin-mediated vasoregulation was critically dependent on activation of 5'AMP-activated protein kinase (AMPK) in the vascular endothelium. In conclusion, the balance of insulin's vasodilator and vasoconstrictor effects in the muscle microcirculation is differentially regulated by the adipokines adiponectin, palmitic acid and TNF . This interaction is dependent on a balance of AMPK, JNK and PKCθ in microvascular endothelium.

S16D

IMPAIRED INSULIN SIGNALLING IN VASCULAR SMOOTH MUSCLE IN INSULIN RESISTANCE

M Trovati

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Arterial Vascular Smooth Muscle Cells (AVSMC) are target of insulin action and site of insulin resistance. In these cells, insulin activates the complex cascade of its signalling, subdivided in the main pathways of phosphatidylinositol-3-kinase (PI3-K) and Mitogen Activated Protein Kinase (MAPK). It is classically stated that: i)PI3-K pathway mediates the vascular anti-atherogenic actions of insulin (i.e. nitric oxide synthesis), whereas the MAPK pathway mediates its pro-atherogenic actions (i.e. AVSMC proliferation); ii)in the insulin-resistant states there is a "selective" resistance for the insulin signalling through the PI3-K pathway, with an unbalance in favour of MAPK pathway, further amplified by the compensatory hyperinsulinemia occurring "in vivo". In our laboratory, we demonstrated in AVSMC that the two pathways cooperate for relevant insulin actions (i.e. synthesis and secretion of Hypoxia-Inducible Factor and Vascular Endothelial Growth Factor), that in the classical genetic model of the obese Zucker rat there is a similar resistance for the insulin-induced activation of PI3-K and MAPK pathways, and that substances increased "in vivo" in the insulin-resistant states, such as free fatty acids, induce a selective resistance at the PI3-K pathway level. Thus, the two pathways should not be simply considered "good" and "bad", and "insulin resistance" should be considered a multifaceted biological phenomenon.

Wednesday, August 27th
Microcirculation in obesity – novel aspects
Parallel Symposium – S17

S17A
FLOW CONTROL IN EXERCISE
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In skeletal muscle oxygen demand and blood flow are closely matched indicating very precise regulatory mechanisms. During exercise blood flow to working muscle can be increased 20-fold, an increase that to a large extent is mediated by several locally formed vasodilators that interact. The identity of vasodilators and their interactions can be examined in humans in vivo by monitoring muscle blood flow during pharmacological inhibition of single and multiple vasodilator systems. Such studies have revealed that there is an interaction between the nitric oxide synthase system and the cyclooxygenase system as well as between Cytochrome P450 2C9 and the nitric oxide system. Moreover, plasma ATP and adenosine both induce a release of nitric oxide and prostaglandins and evidence point at that the primary role of adenosine in exercise hyperemia is via an effect on adenosine receptors on the endothelium, leading to release of nitric oxide and prostaglandins, whereas the direct effect of adenosine on the adenosine receptors on smooth muscle cells appear to be of less importance. Thus, several vasodilators of importance for muscle blood flow control have been identified and evidence point at that the precise regulation of flow is the result of a close interplay between these vasodilators.
Support: Danish Medical Research Council, Novo Nordisk Foundation, Lundbeck Foundation

S17B
EFFECTS OF CHRONIC EXERCISE ON INSULIN-MEDIATED ENDOTHELIAL FUNCTION IN OVARIECTOMIZED RATS
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Postmenopause is characterized by a deficiency of cardioprotective estrogen, which subsequently leads to cardiovascular dysfunction. It is known that insulin regulates cardiovascular function, particularly inducing vasorelaxation through the production of endothelium-derived nitric oxide (EDNO). The purpose was to investigate the influence of chronic exercise on insulin-mediated vasorelaxation in the postmenopause status. Female Sprague-Dawley rats underwent bilateral ovariectomy at 15-weeks old. Rats were randomly assigned to three groups, i.e., sham, ovariectomized (OVX), and OVX with exercise (OVX+Ex) groups. After 10 weeks, the aortas were dissected for the vasorelaxant analysis. Roles of phosphatidylinositol 3-kinase (PI3K) and NO synthase (NOS) in the insulin-induced vasorelaxation were determined by treating selective inhibitors. Additionally, the vascular response to sodium nitroprusside (SNP), a NO donor, was evaluated. We found that, 1) insulin-induced vasorelaxation of the OVX group was markedly lower than that of the sham group ($P<0.05$); 2) chronic exercise significantly improved insulin-induced vasorelaxation in the OVX+Ex group ($P<0.05$); 3) the altered release of PI3K and NOS was found to be mainly involved in the exercise effects; 4) there was no significant difference in the SNP-induced vasorelaxation among three groups. Our findings support the positive effects from chronic exercise on postmenopause-altered vascular function. Supported by grants from the National Sciences Council NSC 95-2314-B-006-073- MY2, Taiwan, ROC.

S17C
EXERCISE-INDUCED ARTERIOGENESIS IN EXPERIMENTAL PERIPHERAL ARTERIAL INSUFFICIENCY
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Peripheral arterial insufficiency is a progressive degenerative disease that affects a large number of adults. Enhanced physical activity is one of the most effective means of improving exercise tolerance, ability for daily activities, and overall outlook on life of affected patients. Meaningful adaptations are induced by exercise, which contribute to this improvement. Evidence from preclinical studies indicates that both conduit vessel and microvascular function can be enhanced, coincident with an upregulation of angiogenic growth factors and their receptors. The increased blood flow (BF) is a major stimulus for vascular remodeling, especially in the enlargement of preexisting collateral vessels where hypoxia is not evident. This vessel enlargement (angiogenesis) requires an effective NO signaling, as L-Name eliminates the exercise-induced in collateral BF; however, angiogenesis within the active ischemic muscle remains to enhance capillarity. Inhibition of VEGF R kinase activity disrupts the increase in collateral development but only tempers the increase in muscle capillarity. The marked improvement in collateral BF with exercise in preclinical studies is not uniformly observed in patients, even though virtually all clinical studies demonstrate a benefit of exercise in patients with intermittent claudication. Whether this is due to an inability to form significant collateral vessels, or an inadequate stimulus for their formation, induced by the exercise program, is not known. Further, distinct hemodynamic limitations in the development of a robust collateral circuit exist in humans. Nonetheless, meaningful vascular remodeling is possible in patients with peripheral arterial insufficiency.

Wednesday, August 27th
Homocysteine and vascular disease
Parallel Symposium – S18

S18A

CARDIOPROTECTIVE ROLE OF SODIUM THIOSULFATE ON CHRONIC HEART FAILURE BY MODULATING ENDOGENOUS H_2S GENERATION

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Sodium thiosulfate (STS) has been shown to be an antioxidant and calcium solubilizer, but the possible role of STS in dysfunctional ventricle remains unknown. Here, we assessed the effects of STS in failing heart. Heart failure was created by arterio-venous fistula (AVF). Mice were divided into four groups: Sham, AVF, Sham + STS and AVF + STS. STS (3 mg/ml) was supplemented with drinking water for 6 weeks in the appropriate post-surgery groups. M-mode echocardiograms showed ventricular contractile dysfunction with reduced aortic blood flow in AVF mice, whereas STS treatment prevented the decline in cardiac function. Ventricular collagen, MMP -2, -9 and TIMP-1 were robustly increased with a decreasing trend in AC_{VI} expression; however, STS supplement reversed these effects in AVF mice. Among two enzymes which produce endogenous H_2S, the CSE (cystathionine-gamma-lyase) expression was attenuated in AVF mice with no changes in CBS (cystathionine-beta-synthase) expression. In addition, reduced production of H_2S in AVF ventricular tissue was normalized with STS supplement. Moreover, cardiac tissues were more responsive to H_2S when AVF mice were supplemented with STS compared to AVF alone. These results suggest that STS modulate cardiac dysfunction and extracellular matrix, in part, by increasing ventricular H_2S generation.

S18B

COX-2 DERIVED PROSTAGLANDIN H2/THROMBOXANE A2 (PGH2/TXA2) MEDIATES FLOW DEPENDENT CONSTRICTIONS OF GRACILIS MUSCLE VENULES IN HYPERHOMOCYSTEINEMIA (HHCY)

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In several venular diseases, the plasma level of homocystein (Hcy) has been shown to be increased. Yet, the effect of HHcy on the vasomotor function of venules has not been established. We hypothesized that HHcy alters the flow dependent regulation of vasomotor tone of venules. Thus, changes in diameter of pressurized venules (active diameter: 250+/-30 microm. at 10 mmHg) isolated from gracilis muscle of normal and HHcy (~5 weeks methionine diet, plasma level of Hcy: ~40 micromol/liter vs. ~6 micromol/liter) rats to increases in perfusate flow were measured. Increases in intraluminal flow elicited dilations of control vessels, whereas in HHcy venules flow resulted in significant constrictions. The PGH2/TXA2 receptor antagonist SQ 29,548 reversed flow-induced constrictions to dilations in HHcy venules. In the presence of SQ 29,548, the NO synthase inhibitor L-NAME and the non-selective cyclooxygenase inhibitor indomethacin (INDO) decreased flow-induced dilations, whereas in their simultaneous presence the flow induced dilations were abolished. The selective cyclooxygenase-2 (COX-2) inhibitor, NS 398 reversed the flow-induced constrictions to dilations, which were significantly decreased by the cyclooxygenase-1 (COX-1) inhibitor, SC 560. Immunohistochemistry showed the presence of both COX-1 and COX-2 in the venular wall. Thus in HHcy the role of COX-2 derived PGH2/TXA2 in mediation of flow-induced responses of skeletal muscle venules is increased. These changes in HHcy could impair the regulation of blood flow and favour increased thrombus formation in the venous circulation. Grants: AHA NE Aff. 0555897T; Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006.

S18C

MEASUREMENT OF HOMOCYSTEINE IN MICRO-SAMPLES

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A protocol for measuring total plasma homocysteine concentration from very small samples is described. The test is based on a selective, recombinant homocysteine , -lyase (rHCYase) and a small portable fluorescence reader. The rHCYase produces hydrogen sulfide (H_2S) from tHCY without interference from physiological range of cysteine or other plasma components. H_2S is determined through reactivity with dibutyl phenylene diamine (DBPDA), which leads to the formation of

a fluorescent chromophore. Only 5 L of plasma/serum sample is required, which can be obtained from a finger prick, suggesting great potential for mass screening. The assay takes approximately twenty minutes.

S18D

HOMOCYSTEINE TO HYDROGEN SULFIDE IN RENAL FAILURE

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Elevated levels of homocysteine (Hcy) are frequently observed in patients with end-stage renal disease (ESRD). Hcy is metabolized in the body to produce an endogenous gaseous substance, hydrogen sulfide (H_2S). Despite its noxious background, recent findings suggest a cardioprotective role of H_2S; however, the potential role of H_2S in Hcy-induced ESRD remains untouched. We tested the hypothesis that H_2S is renoprotective against Hcy-induced deleterious effects on glomerular architecture and function. Genetic model of hyperhomocysteinemia (hHcy) {cystathionine-beta-synthase heterozygous, CBS(+/-)} and wild type (C57/BL 6J) mice were used in this study and supplemented with or without NaHS (H_2S donor) in the drinking water. To expedite the Hcy-induced glomerular damage uninephrectomized (1-K) mice were also used. Plasma Hcy was elevated in CBS(+/-) and 1-K mice; whereas, kidney tissue H_2S was attenuated. Impaired GFR in both CBS(+/-) and 1-K mice were ameliorated by exogenous H_2S administration. Significant increase of collagen, MMP-2, -9, TIMP-1, p47phox, ICAM-1, VCAM-1 were observed in CBS(+/-) and 1-K mice; however, these increases were attenuated by H_2S treatment. Our results suggest that H_2S modulates ROS production, GFR, cortical protein collagen, MMP-2, -9 and TIMP-1 in Hcy-induced renal failure. Additionally, H_2S reduces Hcy-induced NAD(P)H oxidase (p47phox subunit) and acts as an anti-inflammatory substance by modulating inflammatory molecules, ICAM-1 and VCAM-1, in the kidney.

PL3

THE COUNTERINTUITIVE MICROVASCULAR BENEFITS OF INCREASING PLASMA VISCOSITY AND MAINTAINING BLOOD VISCOSITY: EXPERIMENTAL AND CLINICAL FINDINGS

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Intentionally or accidentally decreasing blood viscosity is supposed to be beneficial while the perception that increased blood viscosity promotes or is a consequence of diseases is embodied in the literature. However, increasing blood viscosity in the healthy organism increases shears stress and NO production, causing vasodilatation. Changing blood viscosity linearly affects peripheral resistance, having a highly non-linear opposite effect on the corresponding anatomical component. A microvascular consequence is that blood oxygen carrying capacity can be lowered beyond established transfusion triggers if plasma viscosity is increased, since vasodilatation maintains functional capillary density and increases microvascular flow due to the lowered blood viscosity. Hemorrhagic shock resuscitation improves using high viscosity plasma expanders, and hypertonic-hyperviscous resuscitation is more effective than hypertonic-hyperoncotic resuscitation. Moderate, isovolemic increases of hematocrit cause hypotension.In the normal population elevated hematocrit causes a non-statistical elevation of blood pressure (MAP). The MAP vs. hematocrit distribution in diabetic type 2 patients is "U" shaped and treatment of these patients increases hematocrit and lowers glucose and blood pressure. Treatment of hypertension with diuretics increases hematocrit and lowers blood viscosity. Thus increased or maintained blood/plasma viscosity has a counter intuitive, potentially beneficial microvascular effect that translates into a health benefit.

Marcos Intaglietta
Professor, Ph.D., M.D.
Chairman, Board of Directors
La Jolla Bioengineering Institute
La Jolla, California

Development of effective blood substitutes based on a deep understanding of the microcirculation. Applications to transfusion and emergency medicine, cancer, ischemia, and the heart-lung machine.

Professor Intaglietta has largely influenced present understanding of the effects of viscosity and colloid osmotic pressure on the local regulation of blood flow and oxygen transport at the level of microscopic blood vessels. He is the author or co-author of more than 200 scientific papers and books and pioneered instruments currently in use in laboratories worldwide for studying the microcirculation. His fundamental discovery that oxygen is delivered in small pulses to the microcirculation has provided important information towards creating effective blood substitutes. Previously, substitutes actually caused blood vessels to constrict and shut down because the substitutes delivered too much oxygen.

Intaglietta has also collaborated for many years with international colleagues in Europe, Latin America and Asia on projects related to blood plasma expanders for use in emergency situations and for companies with low blood bank supplies.

Marcos Intaglietta joined UCSD in 1966 as a founding bioengineering faculty member. His pioneering work on artificial blood substitutes with Robert Winslow is now being developed through Sangart, a San Diego based company. He is the Chair of Sangart's Scientific Advisory Board, and serves on the company's board of directors. Intaglietta received his Ph.D. in mechanical engineering from the California Institute of Technology in 1963. He founded the International Institute for Microcirculation, and is an honorary member of the Italian, French, Indian, and Mexican societies for microcirculation. Intaglietta received the Malpighi Gold Medal Award from the European Society for Microcirculation (1994), the Whitaker Award (1996) and the International Award (2002) from the Biomedical Engineering Society, and the Landis Award (1999) from the Microcirculation Society.

Thursday, August 28th
Nitric oxide, NADPH oxidases, mitochondria and oxidative stress I.
Parallel Symposium – S19

S19A
REACTIVE OXYGEN SPECIES AND NAD(P)H REDOX REGULATION OF VASCULAR RESPONSES TO HYPOXIA AND NITRIC OXIDE-cGMP SIGNALING
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Reactive oxygen species (ROS) and cytosolic NAD(P)H redox potentially influence vascular responses elicited by changes in oxygen tension and nitric oxide (NO). Hypoxia relaxes bovine coronary arteries through a pyruvate-modulated mechanism, which oxidizes cytosolic NADPH. Elevated levels of glucose-6-phosphate dehydrogenase and NADPH in bovine pulmonary arteries maintain a tonic relaxation by increasing superoxide-derived peroxide from Nox oxidase. Cu,Zn-SOD activity enables the generation of peroxide, which mediates relaxation removed under hypoxia, leading to hypoxic pulmonary vasoconstriction. Lowering Nox4 (but not Nox2) activity or expression attenuates and increasing Nox4 expression with TGFß1 enhances pulmonary artery contraction to hypoxia. While mitochondria seem to be a source of ROS in both coronary and pulmonary arteries potentially modulated by hypoxia, these changes do not cause the observed responses to hypoxia. For example, hypoxia appears to increase mitochondrial NADH and superoxide in coronary arteries, but contraction with 30 mM KCl attenuates these changes. Stimulation of soluble guanylate cyclase and cGMP-mediated relaxation by NO is known to be attenuated by superoxide and heme oxidation. In addition, cytosolic NADPH appears to maintain reduced thiols and heme sites on sGC needed for its stimulation by NO. Thus, NAD(P)H redox and ROS regulate vascular responses to hypoxia and NO. These studies were supported by NIH grants HL31069, HL43023 and HL66331.

S19B
MECHANISMS, REGULATION AND CONSEQUENCES OF FREE RADICAL GENERATION IN THE CNS
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We have explored the mechanisms that generate reactive oxygen species (ROS) in neuronal and glial cells in culture following deprivation of oxygen and glucose (OGD) or the application of neurotoxic amyloid- peptides. Rates of ROS generation are measured by confocal fluorescence imaging using the dye Dihydroethidium. ROS generation in response to OGD engages several distinct mechanisms in a sequence that reflects the temporal evolution of changes in mitochondrial membrane potential, rates of ATP depletion, changes in $[Ca^{2+}]_c$ and activation of the NADPH oxidase. These processes each dictate progressive changes in ROS generation with time. Amyloid- peptides activate the NADPH oxidase in astrocytes and microglia. In astrocytes this causes oxidative stress, activation of PARP, loss of mitochondrial membrane potential, and cell death. Microglial ROS generation by the NADPH oxidase in response to Amyloid- is modulated by the redox dependent activation of CLIC1, a chloride conductance that provides charge compensation for the electrogenic activity of the enzyme. Our data imply that rates of ROS generation in response to a number of stimuli will differ between cell types according to variations in the underlying processes between cells. Supported by the Wellcome Trust.

S19C
NADPH OXIDASES: SIGNAL TRANSDUCTION THROUGH REACTIVE OXYGEN SPECIES
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Reactive oxygen species (ROS) are central effectors of the vascular homeostasis. By activating or modulating the cellular signal transduction, ROS can elicits such different responses as induction of redox-sensitive genes, apoptosis, proliferation, hypertrophy, migration and differentiation. Nox family NADPH oxidases are the predominant sources of ROS and isoforms are differentially expressed in the vasculature. Among those proteins, Nox4, which is ubiquitously expressed, generates ROS constitutively and thus determines the basal cellular ROS balance. Nox4 appears to control cellular activity state and thus Nox4 acts as a switch between resting, differentiating and activated, proliferating cells. The other two NADPH oxidases expressed in the vasculature generate ROS in an agonist-dependent manner, usually as small bursts of ROS. Through such a mechanism, Nox1 in smooth muscle cells and Nox2 in endothelial cells facilitate agonist-induced signalling of important growth factors such a VEGF or angiotensin II. Continuous exposure to stimuli in scenarios like diabetes, hypertension or hyperlipidemia however further induce Nox1 and Nox2 which then results in oxidative stress. By limiting NO availability and activating pro-inflammatory transcription factors, NADPH oxidases then promote vascular disease initiation and progression.

S20A
INTERACTIONS AMONGST INTRALUMINAL PRESSURE, ARTERIOLAR TONE AND CA^{2+} OSCILLATIONS
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Arteriolar smooth muscle cells exhibit local variation in intracellular Ca^{2+} levels as shown by phenomena including sparks, flashes and waves. The exact relationship of such events to levels of intraluminal pressure and myogenic tone, however, remains unclear. Using Fluo 4 (10 uM) loaded cannulated rat cremaster arterioles these studies examined variations in cytosolic Ca^{2+} levels. Changes in fluorescence were measured using a spinning disk confocal system coupled to an inverted microscope (x60, 1.2 N.A. objective) and a high speed intensified CCD camera. Measurements of Ca^{2+} were collected at 30-60Hz and regions of interest defined over single cells (3-5 cells/ vessel) of the vessel wall. Measurements were taken at 10, 40, 70 and 100 mmHg under baseline conditions and following treatment with either nifedipine (1 uM) or adenosine (10uM). Under control conditions, increasing intraluminal pressure was associated with a significant increase in the number of cells exhibiting oscillations and an increasing frequency. These changes with pressure persisted following either nifedipine or adenosine. In contrast to active constriction observed at baseline, arterioles exposed to nifedipine or adenosine responded passively to pressure increases. Pressure dependency of Ca^{2+} waves was abolished by ryanodine but not 2APB. Thus, oscillations in intracellular Ca^{2+} interact with pressure-induced signaling events and involve ryanodine-sensitive Ca^{2+} stores, but are not dependent on active myogenic constriction.

S20B
ROLE OF THE ENDOTHELIUM ON ARTERIAL VASOMOTION: EXPLANATION ON CONTRADICTORY EFFECTS
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Cyclic variations of the vascular diameter (vasomotion) are induced by synchronous calcium oscillations of smooth muscle cells (SMCs). Contradictory results have emerged in the literature about the role of the endothelium in the onset and maintenance of vasomotion. By modeling the calcium dynamics of SMCs coupled to a population of endothelial cells, we show that the endothelium essentially decreases the SMCs calcium level and may move the SMCs from a steady state to an oscillatory domain, and vice versa. In the oscillatory domain, a population of coupled SMCs exhibits synchronous calcium oscillations. Outside the oscillatory domain, the coupled SMCs present only irregular calcium flashings arising from noise modeling stochastic opening of channels. To test this model, we investigate rat mesenteric arterial strips stimulated with phenylephrine (PE). As predicted by the model, the endothelium is not necessary for vasomotion. Endothelium-derived relaxing products may either induce or abolish vasomotion. When the strip is tonically contracted, an endothelium-derived relaxation may induce vasomotion. When the strip displays vasomotion, a relaxation may induce a transition to a non-oscillating slightly contracted state. Our findings clarify the role of the endothelium on vasomotion and reconcile the seemingly contradictory observations reported in the literature. This work was supported by the Swiss National Science Foundation (FN 3152-067939 and FN 310000-114097).

S20C
Ca^{2+} PUFFS AND WAVES IN ARTERIAL ENDOTHELIAL CELLS
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Increases in global Ca^{2+} in the endothelium are a crucial step in releasing relaxing factors to modulate arterial tone. In pressurized arteries, high-speed fluorescence microscopy shows that spontaneous Ca^{2+} events occur in both endothelial cells and smooth muscle cells. The endothelial cell spontaneous Ca^{2+} events depend on the activity of phospholipids C, and are influenced by the levels of Ca^{2+} in the adjacent smooth muscle cells, the latter via the passage of signals through myoendothelial gap junctions. Increasing the luminal pressure causes an increase in spontaneous Ca^{2+} events in smooth muscle cells, yet at the same time, causes a decrease in spontaneous Ca^{2+} events in endothelial cells. This apparent anomaly cannot be explained by pressure-dependent changes in cellular membrane potential or diameter. Possible alternate explanations will be discussed.

S20D
MODULATION OF GAP JUNCTIONAL COMMUNICATION BY NITRIC OXIDE
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Gap junctions (GJ) allow for communication (GJC) between endothelial and /or smooth muscle cells. We tested whether NO acts as a regulator of GJC. The GJ dependent spreading of small charged dyes such as Alexa 488 (resembling cyclic

nucleotides) was significantly reduced by the NO donor SNAP in HUVEC, an effect that was abolished in the presence of phosphatase inhibitors. In Cx-transfected HeLA cells, SNAP specifically reduced the conductivity of Cx37 containing GJ in a cGMP independent manner. The propagation of mechanically induced calcium waves was also reduced by NO. Whereas SNAP only reduced the propagation velocity of the calcium wave in control cells, it significantly reduced the propagation distance of the calcium wave when HUVEC had been pretreated with an siRNA against Cx43. These results are consistent with the hypothesis that NO specifically inhibits Cx37-dependent GJ communication, such as exchange of small molecules and propagation of calcium waves. This effect may be masked in endothelial monolayers by compensatory propagation via GJ formed by Cx43. A pronounced effect of NO on GJ communication is to be expected in gap junctions where Cx37 is enriched, such as it was reported for some myoendothelial junctions. Supported by DFG PO 414/2-1

S20E

CHARACTERISATION OF Ca^{2+} SIGNALLING IN SMOOTH MUSCLE CELLS AND PERICYTES OF THE RAT URETERIC PRECAPILLARY SPHINCTERS

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Although it has long been recognized that the control of the blood flow in capillaries is controlled by “precapillary sphincters” (PS), it is unclear what type of cells perform this important physiological function. Smooth muscle cells (SMC) originating from precapillary arterioles (PA), precapillary pericytes expressing contractile proteins and endothelial cells have all been suggested to perform a “sphincter” function. So far, no study has investigated mechanisms of generation and properties of Ca^{2+} signalling in SMC and precapillary pericytes of PS *in situ*. In the present study we used high-resolution X-Y-Z and fast temporal confocal imaging of *in situ* ureteric microvessels loaded with the Ca^{2+}- sensitive indicator Fluo-4 in order to investigate morphology, Ca^{2+} signalling and mechanical activity of SMC and precapillary pericytes comprising ureteric PS (inner diameter, i.d.<10μm). The effects of central (phenylephrine, PhE) and local (endothelin-1, ET-1) factors as well as caffeine - a powerful vasoconstrictor on Ca^{2+} signalling and vessel activity in PA (i.d.=15-25μm) and PS (i.d.<10μm) have been investigated.

Two types of PS have been defined in ureteric microvascular bed *in situ*: type 1 (length >40μm) which contained both SMC and precapillary pericytes running circumferentially around the endothelium; and type 2 (length <40μm) in which the endothelium was encircled only by a group of precapillary pericytes. Each SMC normally made 3 full turns around the endothelium and occupied a segment of 8.18±0.21μm (n=12). Precapillary pericytes formed an asymmetrical coat with a thick massive body located on one side of the vessel giving 2-3 finger like processes, which tightly wrapped around the endothelium. Each precapillary pericyte occupied a segment of 10.10±0.48μm (n=15). SMC of PA responded with ryanodine - resistant $[Ca^{2+}]_o$ – independent asynchronous Ca^{2+} oscillations when stimulated by either 10 μM PhE (0.035±0.003 Hz) or 10nM ET-1 (0.046±0.004 Hz) and ryanodine sensitive Ca^{2+} sparks or Ca^{2+} waves when stimulated by low (0.5-1mM) or high (2-5mM) concentrations of caffeine, respectively. In marked contrast, SMC of PS were insensitive to caffeine (1-10mM) and PhE (10-100μM) but readily responded to ET-1 with ryanodine resistant and $[Ca^{2+}]_o$ – independent asynchronous Ca^{2+} oscillations (0.068±0.005 Hz). In both PA and PS agonist induced Ca^{2+} oscillations resulted in generation of regional oscillatory vasoconstriction. Precapillary pericytes were insensitive to both caffeine (10mM) and PhE (10-100μM) but responded to ET-1 (10nM) with a non-oscillatory ryanodine resistant $[Ca^{2+}]_o$ – independent Ca^{2+} transient which consisted of an initial fast followed by a slowly decaying sustained component associated with a generation of a strong “grip-like” constriction of PS. The data indicate that there is a marked difference in the mechanisms controlling Ca^{2+} signalling and mechanical activity between SMC and precapillary pericytes of the ureteric PS. Also, the data obtained suggest that the latter are more likely to act as local sphincters controlling capillary blood flow responding to local factors.

Thursday, August 28th
Coronary microcirculation in heart failure
Parallel Symposium – S21

S21A

MECHANISM OF CARDIOVASCULAR MORTALITY DURING LOW SODIUM DIET: NO BIOAVAILABILITY AND THE RENIN ANGIOTENSIN.NADPH OXIDASE SYSTEM

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Adult male mongrel dogs were placed on low sodium diet (LS) which contains 0.5% sodium chloride for two weeks. Body weight (25±0.4kg to 24±0.4kg), left ventricular systolic pressure (137±3.4 to 124±6.7mmHg) and mean aortic pressure (MAP) (111±3.1 to 98±4.3 mmHg) decreased. Plasma angiotensin II concentration increased (4.4±0.7 to 14.8±3.7pg/ml), and losartan (10mg/kg) decreased MAP (95±4.7 to 52±4.1 mmHg) in LS dogs. In LS heart tissues, lucigenin chemiluminescence was increased 2.3 fold to angiotensin II. Bradykinin induced reduction of MVO in vitro was decreased (-40±1.3 to -16±6.3%). These changes were completely restored by coincubation with tiron, tempol (10^{-3} M each), or apocynin (10^{-4} M. Veratrine (5µg/kg) induced NO- dependent coronary vasodilation was inhibited by 44%, but the simultaneous intravenous infusion of ascorbic acid or apocynin completely reversed this inhibition. Switching of cardiac substrate uptake from free fatty acid (FFA) to glucose by the heart was observed (FFA; 8.97±1.39 to 4.53±1.12 µmol/min, Glucose; 1.31±0.52 to 6.86±1.78 µmol/min). Western blotting analysis showed that the protein expression of $p47^{phox}$ was upregulated in the LS heart tissue. In conclusion, LS diet induces the activation of the renin-angiotensin system which increases oxidative stress via the NADPH oxidase, and attenuates the NO bioavailability in the heart.

S21B

CORONARY MICROVASCULAR DYSFUNCTION IN DILATED CARDIOMYOPATHY

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There is growing evidence of the presence and relevance of coronary microvascular abnormalities in many cardiac diseases. In particular, it has been recently shown that dilated cardiomyopathy (DCM) is characterized by dysfunction of the coronary microvessels since its very early onset. Coronary microcirculatory dysfunction is not an effect of myocardial damage but seems in turn to cause progressive contractile impairment, ventricular dilation and heart failure. The mechanisms of the progressive deterioration of cardiac function in DCM are largely unknown but both myocardial hypoperfusion and myocardial ischemia at the microvascular level are most probably involved. It has been demonstrated that the presence and the extent of coronary microcirculatory dysfunction in patients with early stage DCM is an undependent and relevant predictor of worse prognosis. From these studies it is more and more evident that the coronary microcirculation is involved in the pathogenesis of DCM and should be considered a new target of treatment in those cardiac diseases at risk to evolve towards heart failure.

S21C

DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN TRANSGENIC MOUSE MODEL OF DILATED CARDIOMYOPATHY

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Tgαq*44 mice that mimic phenotype of human dilated cardiomyopathy display a protracted time of the development of HF with compensated stage till 10-12 months of age and a transition to decompensated state and the progression to end-stage heart failure in 12-18 month-old mice (Mende et al 2001, JMCC, 33 (8), 1477, Drelicharz et al 2008, BRC, in press. Elas et al 2008, FRBM in press). We analyzed development of pathology in Tg□q*44 mice on morphological, molecular and functional levels. In particular we characterized the progression of the impairment of cardiac function by MRI and endothelial function including assessment of NO-, PGI_2- and EDHF-dependent pathways at early (2-8 month of age), and later phases (12-16 months of age) of heart failure development in the coronary circulation (the isolated heart set-up) and in aorta (aortic rings).
In Tgαq*44 mice at the age of 2-8 months cardiac function was preserved and endothelial function unchanged. In contrast, in Tgαq*44 mice at the age of 14-16 months cardiac function was significantly impaired and NO-mediated endothelial function was decreased in both the coronary circulation and the thoracic aorta. Basal production of COX-1 derived PGI_2 was increased in 14-16 months old Tgq*44 hearts, but not in the thoracic aorta. Neither in coronary circulation nor in aorta EDHF pathway was upregulated. In conclusion, late development of endothelial dysfunction could be an important factor that contribute to the progression to over heart failure in Tgαq*44 mice. Differential phenotype of endothelial dysfunction in coronary resistance vessels and peripheral conduit vessels underscore the important heterogeneity of endothelial response in heart failure. The secondary type of endothelial dysfunction in Tgαq*44 mice also differs with the phenotype of primary endothelial dysfunction that develops in atherosclerotic ApoE/LDLR-/- mice.

S21D

MICROVASCULAR FUNCTION IS IMPAIRED IN PEOPLE WITH SYMPTOMATIC CORONARY ARTERY DISEASE AND PREDICTS SYMPTOMS INDEPENDENT OF CORONARY ATHEROSCLEROSIS

WD Strain [1], N Chaturvedi [2], J Kooner [3], A Wright [4], SM Thom [2], J Mayet [2], AD Hughes [2], AC Shore [1]

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Coronary artery disease (CAD) is the biggest killer in the UK, however, there is poor correlation between symptoms and coronary angiographic stenosis. 83 men with angiographic ally confirmed atherosclerotic CAD and 84 age-matched controls underwent CT coronary artery calcification scoring, a non-interventional quantitative measure of coronary atherosclerosis, and skin laser Doppler fluximetry in response to heating and ischaemia, measuring microvascular function. CAD symptoms were assessed by the Rose angina questionnaire. Men with CAD symptoms had poorer microvascular function than asymptomatic men (maximum and reactive hyperaemia 114(95% CI 106-122) vs. 143(134-153) arbitrary units (au) $p<0.001$ and 42(38-46) vs. 53(48-58)au; $p=0.001$ respectively). This difference was unaccounted for by conventional cardiovascular risk factors or coronary artery calcification scores ($p=0.02$ & 0.001 after adjustment). Thirty-eight patients had persistent angina despite coronary intervention. These individuals had poorer microvascular function compared to those with CAD without angina (109(95-124) vs. 134(127-141); $p=0.0007$ and 40(45-53) vs. 49(45-53); $p=0.01$ respectively). The only independent predictors of persistent angina were reactive hyperaemia, fasting insulin and diastolic blood pressure ($p=0.009$, 0.005 and 0.03 respectively). Symptomatic CAD is associated with microvascular dysfunction independent of conventional CAD risk factors or coronary atherosclerosis. Therapies that improve microvascular function may improve symptoms in patients with angina. We would like to thank the British Heart Foundation for their support of this study.

Thursday, August 28th
Nitric oxide, NADPH oxidases, mitochondria and oxidative stress II.
Parallel Symposium – S22

S22A
NOX FAMILY OF NADPH OXIDASES: HOST DEFENSE AND MORE
M Geiszt
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Reactive oxygen species (ROS) play a central role in many physiological processes including host defense, hormone biosynthesis, fertilization and cellular signalling. Altered production of ROS has been implicated in the development of immunodeficiency, hypothyroidism and cardiovascular pathologies. In the last few years, several enzymes were identified at the molecular level, which are now thought to be responsible for ROS production observed in diverse tissues. These enzymes show a high degree of homology to the phagocytic NADPH oxidase and are now designated the Nox family of NADPH oxidases. In my lecture I will discuss our current knowledge on six new members of the Nox family, with a special emphasis on their possible role in host defense.

S22B
NO AND SUPEROXIDE: INTERFERENCE WITH HYPOXIC SIGNALING
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Hypoxia inducible factor-1 (HIF-1) is a regulator of metabolic adaptation to hypoxia. It is appreciated that accumulation of HIF-1α is achieved under normoxic conditions by nitric oxide (NO). Mechanistically, this is explained by inhibition of PHD (prolyl hydroxylase) activity, an attenuated interaction with the E3 ubiquitin ligase pVHL (von Hippel Lindau protein), decreased ubiquitination of HIF-1α and subsequently impaired proteasomal degradation. In contrast, hypoxic stabilization of HIF-1α can be antagonized by NO due to its ability to attenuate mitochondrial electron transport. Likely, formation of reactive oxygen species (ROS) may contribute to this effect. Mimicking ROS changes by employing the redox cycling agent 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), we concentration-dependently raised intracellular ROS, which correlated with HIF-1α accumulation. Using a HIF-1α-pVHL binding assay, we showed that ROS produced by DMNQ impaired PHD activity. When HIF-1α is stabilized by NO, low concentrations of DMNQ (< 1 μM) revealed no effect, intermediate concentrations of 1 to 40 μM DMNQ attenuated HIF-1α accumulation, whereas with higher amounts of DMNQ HIF-1α stability reappeared. Attenuating NO-induced HIF-1α stabilization by ROS required an active proteasomal degradation pathway, which implies that scavenging of NO by ROS and vice versa limits HIF-1α accumulation.

S22C
OXIDATIVE STRESS, INFLAMMATION AND ATHEROSCLEROSIS – FOCUS ON MICROVASCULAR ANGINA
JC Kaski
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Oxidative stress has been implicated in the pathogenesis of cardiovascular disease and there is an important link to diabetes, dyslipidaemia, hypertension, and central obesity. Several factors can impair NO formation leading to endothelial dysfunction, including hyperglycaemia, oxLDL, ROS, and asymmetric dimethylarginine (ADMA). eNOS catalyses NO production but eNOS 'uncoupling' can occur in states associated with eNOS substrate (L-arginine) or co-factor (tetrahydrobiopterin, BH4) deficiency, producing ROS. ADMA can initiate and promote atherogenesis via impaired eNOS pathways, increased ROS generation and enhanced endothelial adhesiveness for monocytes. Coronary microvascular dysfunction may be responsible for chest pain and myocardial ischaemia in a broad spectrum of angina syndromes. Cardiac syndrome X (CSX) (chest pain with normal coronary arteriograms) represents one end of the spectrum where patients develop myocardial ischaemia in the absence of obstructive coronary artery disease or coronary vasospasm. CSX patients have abnormal vasodilator responses to pharmacologic agents and exercise. We have shown that inflammation modulates microvascular dysfunction and angina in CSX patients. Elevated C-reactive protein (CRP) levels correlate with clinical presentation, exercise stress test responses, and ST-segment changes during daily life in CSX patients. More recently, we have expanded our observations to patients with angina pectoris, normal coronary arteriograms and chronic systemic inflammatory diseases but without traditional cardiovascular risk factors, in whom coronary microvascular dysfunction appears to be caused solely by the inflammatory process responsible for their background disease. This patient group provides interesting information regarding oxidative stress and inflammation as potential triggers of microvascular angina in the absence of coronary artery disease.

S22D
OXIDATIVE STRESS, LIPID PEROXIDATION, AND ULTRASTRUCTURAL DAMAGE IN PATIENTS WITH MYOCARDIAL ISCHEMIA

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In experimental models, formation of oxidants during postischemic reperfusion may exert deleterious effects, i.e., "oxidative stress". Cardioplegic arrest/reperfusion during cardiac surgery might similarly induce oxidative stress. However, this phenomenon has not been fully characterized in patients, and value of antioxidant therapy during cardiac surgery is still debated. Recently, we have systematically investigated the relationship between oxidant formation and development of myocardial injury during reperfusion after cardioplegic arrest. In patients undergoing elective coronary artery bypass, trans-cardiac blood samples and myocardial biopsies were taken before cardioplegic arrest and following reperfusion. By this approach, we demonstrated that cardiac glutathione release was negligible at baseline, but it increased by several fold into reperfusion: this finding demonstrates that oxidant production takes place during postischemic reperfusion; furthermore, we concomitantly observed that myocardial concentration of the endogenous antioxidant ubiquinol significantly decreased in biopsies taken at reperfusion. However, although these changes do show cardiac exposure to oxidants, they were not accompanied by evidence of injury. Neither coronary sinus blood, nor cardiac biopsies, showed increased lipid peroxidation. Furthermore, electron microscopy showed no major ultrastructural alterations. Finally, full recovery of left ventricular systolic and diastolic function was observed. In conclusion, although oxidant production occurs in patients undergoing cardiac surgery, under conditions encountered in modern days surgery cardiac oxidative stress does not progress through membrane damage and irreversible injury.

Thursday, August 28th
Calcium oscillations and calcium waves in microvascular control
Parallel Symposium – S23

S23A

CALCIUM WAVES UNDERLY THE APPARENT ENDOTHELIAL SENSITIVIY TO VASOACTIVE COMPOUNDS

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To analyse a potential role of calcium waves in endothelial signalling we tested in HUVEC whether vasoactive agonists could stimulate all cells directly or whether gap junctions (GJ) were required for a homogeneous calcium response. Under control conditions, virtually all cells finally responded with a calcium rise after 3 seconds. Inhibition of GJ coupling reduced the number of responding cells to about 40% corresponding well to the percentage of cells expressing the H1-receptor (FACS). Blockade of GJ also significantly reduced the HIS induced NO production. Likewise, blockade of GJ also reduced the endothelium-dependent dilation of isolated resistance arteries. Calcium waves induced by mechanical stimulation of a single cell propagated over three to four rows of adjacent cells. The IP3 receptor blocker Xestospongine nearly abolished any calcium rise beyond the immediately adjacent cell row. It is concluded that the histamine-induced increase of endothelial calcium is only partly a direct receptor induced event. A homogenous calcium response of all cells critically depends on GJ, which suggests a new modulator role of GJ on apparent endothelial sensitivity to vasodilators. GJ dependent calcium wave propagation seems to be partly carried by diffusion of free calcium ions and partly requiring IP3 dependent calcium release. Supported by DFG PO414/-1 and EU- Integrated Project Exgenesis

S23B

CALCIUM WAVES IN LUNG CAPILLARIES: PERMEABILITY AND BEYOND

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Pulmonary microvascular endothelial cells in intact lung preparations but not in cell culture show spontaneous $[Ca^{2+}]_i$ oscillations, which are generated by a subgroup of endothelial cells termed pacemaker cells. These cells are localized at microvascular branch-points, have a higher mean $[Ca^{2+}]_i$ and propagate $[Ca^{2+}]_i$ oscillations to adjacent non-pacemaker endothelial cells via gap junctions. The evolving interendothelial Ca^{2+} waves propagate along the vessel wall and may coordinate microvascular functions.

Activation of endothelial cells by mechanical stress or TNF- amplifies endothelial $[Ca^{2+}]_i$ oscillations and $[Ca^{2+}]_i$ waves by stimulating Ca^{2+} release from endoplasmic stores and secondary mitochondrial Ca^{2+} uptake in endothelial pacemaker cells. Absence of pacemaker-generated spontaneous $[Ca^{2+}]_i$ oscillations is associated with an impaired regulation of microvascular homogeneity, and a reduced microvascular permeability in response to mechanical or pharmacological stimulation. Consistently, inhibition of intercellular Ca^{2+} signal propagation by connexin-mimetic peptides prevents the lung microvascular permeability increase in response to thrombin, and inhibition of gap junctional communications prevents the spread of endothelial apoptosis from brach-point to adjacent cells. These findings suggest a critical role for pacemaker-generated endothelial Ca^{2+} oscillations in the physiological regulation of lung microvascular homeostasis, as well as in the rapid dissemination of microvascular leakage and cell death in acute lung injury. This study was funded by DFG Ku1218/1 and 1218/5.

S23C

ROLE FOR ENDOTHELIAL CALCIUM WAVES IN CONDUCTED VASODILATATION

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Smooth muscle cells (SMC) along the resistance vasculature must relax in concert for tissue blood flow to increase. Whereas the spread of hyperpolarization through gap junctions underlies rapid (cm/s) conducted vasodilation, we investigated a complementary role for calcium waves along the endothelium. Isolated feed arteries of hamster retractor muscle were pressurized (75 mmHg) to develop myogenic tone. Endothelial cells and SMC were loaded with Fluo-4 and $[Ca^{2+}]_i$ was determined using confocal imaging. ACh microiontophoresis triggered local increases in endothelial $[Ca^{2+}]_i$, which preceded two distinct events: rapid synchronous decrease in SMC $[Ca^{2+}]_i$ along the entire vessel segment and ensuing calcium wave that propagated bidirectionally along the endothelium. Local blockade of KCa channels eliminated the rapid response to reveal 'slow-conducted vasodilation' associated with endothelial calcium waves. In cremaster muscles of transgenic mice expressing the calcium sensor 'GCaMP2' selectively in arteriolar endothelium, ACh triggered a robust $[Ca^{2+}]_i$ signal that propagated bidirectionally at ~0.1 mm/s while decaying into oscillations. Rapid dilation occurred along several mm without remote changes in endothelial $[Ca^{2+}]_i$ followed by additional dilation along ~1 mm in the wake of a calcium wave. Respective signaling pathways act to coordinate the onset, extent and duration of vasodilation during blood flow control. (Support: NIH HL41026 & HL56786; MI Kotlikoff & NY Tallini @ Cornell University)

S23D

IN PHASE AND OUT OF PHASE CALCIUM OSCILLATIONS IN ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Vasomotion is associated with synchronized oscillations of calcium in smooth muscle cells. It is uncertain whether this is accompanied with synchronized oscillations in endothelial cells, but unsynchronized calcium transients are seen in endothelial cells exposed to acetylcholine. We have investigated whether it is possible to elicit synchronized calcium oscillations in endothelial cells in isolated segments of rat mesenteric small arteries. Endothelial cells and smooth muscle cells were loaded with calcium sensitive dyes and individual cells visualized with confocal microscopy. Noradrenaline elicited oscillations in tone and synchronized oscillations in smooth muscle cell calcium but little or no oscillation in endothelial cell calcium. After inhibition of the endoplasmic reticulum calcium-ATPase with cyclopiazonic acid noradrenaline induced vasomotion with a large amplitude and low frequency. This oscillation of tone was associated with synchronized oscillations in smooth muscle cell calcium and synchronized, but antiphase oscillations of endothelial cells calcium. We conclude that under conditions with a depleted, nonfunctional endoplasmic reticulum endothelial cell can exhibit large synchronized calcium oscillations probably driven by changes in the membrane potential. The data further suggest a limited diffusion of calcium between smooth muscle and endothelial cells under these conditions.

S24A

A ROLE FOR PERIPHERAL TACHYKININS AND THE NEUROKININ 1 RECEPTOR IN THE REGULATION OF PLATELET THROMBUS FORMATION

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Platelets perform an essential role in haemostosis but also trigger arterial thrombosis, which underlies heart attacks and strokes, and are therefore a principal cause of considerable levels of mortality and morbidity. Understanding of how the functions of platelets are regulated is key to the development of safe and effective means to prevent or treat these conditions. We have reported that platelets are activated by the tachykinin substance P (SP) and have proposed that SP released from platelets during activation mediates positive feedback stimulation. In this study, we define a role for peripherally distributed members of the tachykinin family of peptides, namely SP and the newly discovered endokinins A and B that are present in platelets, in the activation of platelet function and thrombus formation. We have reported previously that the preferred pharmacologically characterised receptor for these peptides, the NK1 receptor, is present on platelets. Inhibition or deficiency of the NK1 receptor, or SP agonist activity, resulted in substantially reduced thrombus formation *in vitro* under arterial flow conditions, increased bleeding time in mice and a decrease in experimentally induced thromboembolism. Inhibition of the NK1 receptor may therefore provide benefit in patients vulnerable to thrombosis and may offer an alternative therapeutic target. This study was supported by research grants from the British Heart Foundation, Medical Research Council, Wellcome Trust and National Centre for the Replacement, Refinement and Reduction of Animals in Research.

S24B

PROTEIN KINASE C ISOFORMS CRITICALLY REGULATE THROMBUS FORMATION: EVIDENCE FROM MOUSE MODEL SYSTEMS

O Konopatskaya [1], K Gilio [2], MT Harper [1], JM Cosemans [2], ZA Karim [3], SW Whiteheart [3], JD Molkentin [4], P Verkade [5], JWM Heemskerk [2], AW. Poole [1].
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Platelets are central players in the atherothrombosis of coronary artery disease. The protein kinase C family provides important intracellular mechanisms for regulation of platelet activity, and platelets express several members of this family including classical isoforms PKC□ and PKC□ and novel isoforms PKC□ and PKC□. Here we use a genetic approach to demonstrate definitively for the first time the predominant role played by the classical isoform PKC□ in regulating thrombus formation and platelet function. Critically, PKC□ is required for thrombus formation when blood is flowed over a collagen-coated surface, although adhesion of platelets to collagen is not regulated by PKC□. Combined genetic and pharmacological approaches revealed that the ablation of thrombus formation in PKC□-/- platelets could be rescued by addition of ADP. This is consistent with the key finding that dense granule biogenesis and secretion depends upon PKC□ expression. In combination with the observation of no overt bleeding disorder seen in these mice, including gastrointestinal bleeding, PKC□ may represent an excellent drug target for antithrombotic therapy, with selective inhibitors potentially exerting a major effect upon thrombus formation whilst sparing primary platelet adhesive functions. This study was supported by research grants from the British Heart Foundation and the Biotechnology and Biological Sciences Research Council.

S24C

ABNORMAL BLOOD RHEOLOGY IN CARDIAC SYNDROME X PATIENTS

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Based on large-scale clinical studies, the prevalence of Cardiac Syndrome X (CSX; microvascular angina) is 10-30% in the general population yet the underlying pathophysiological mechanisms have not been fully elucidated. Elevated whole blood viscosity (WBV) and RBC aggregation are known to adversely affect microcirculatory blood flow; the present study was thus designed to explore whether CSX is associated with abnormalities of blood rheology. Samples were obtained from 152 adult patients prior to diagnostic coronary angiography at the LAC-USC Hospital; 21 subjects were later identified with CSX. Their hemorheological and clinical laboratory results were compared to those of 21 age- and gender-matched healthy

controls with no evidence of coronary disease. CSX patients had markedly abnormal blood rheology: 1) Higher RBC aggregation as judged by ESR and Myrenne aggregometer data at stasis and low shear ($p<0.001$); 2) Elevated hematocrit-corrected blood viscosity ($p<0.001$), plasma viscosity ($p<0.001$), and yield stress ($p<0.01$). Inflammatory markers (i.e., WBC count, hs-CRP) were also significantly elevated in CSX patients; coronary flow velocities were below normal. Our results indicate that abnormal hemorheological parameters exist in CSX subjects and suggest that these abnormalities may contribute to the pathophysiology of the disease, presumably via adversely affecting blood flow in the coronary microcirculation.

S24D

GLOBULAR ADIPONECTIN PROMOTES PLATELET ACTIVATION AND THROMBUS FORMATION UNDER FLOW CONDITIONS

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Adiponectin (Ad) is one of the adipocytokines secreted exclusively by adipose tissue. In circulation Ad exists in full-length and globular isoforms, which may mediate independent biological effects. The biological actions of Ad include anti-diabetic, anti-inflammatory and vascular protective actions, although the influence of Ad on platelet function is unclear. Globular adiponectin (gAd), but not full length Ad, induced rapid aggregation and granule secretion of human and mouse platelets through a tyrosine kinase-dependent mechanism. Consistent with this, gAd stimulates rapid tyrosine phosphorylation of several proteins in human and mouse platelets. The pattern of tyrosine phosphorylation was similar to that induced by collagen. Since collagen activates platelet through the GPVI-Fc receptor □-chain (FcR□) complex, we used FcR□-chain null platelets to explore the mechanism by which gAd stimulates platelets. Stimulation of tyrosine phosphorylation and platelet aggregation by gAd was abolished in FcR□-chain null platelets and markedly reduced in the absence of PLC□2. Further, immobilized gAd induced the formation of whole blood thrombi under arterial shear rates and potentiated the thrombotic effects of von Willebrand factor. These data suggest that gAd is a novel platelet agonist that may promote unwanted platelet activation at sites of vascular injury and contribute to the accelerated atherothrombosis. Research in this laboratory is funded by the British Heart Foundation, Heart Research UK and the Wellcome Trust.

S24E

SEASONAL VARIATIONS IN HEMORHEOLOGICAL PARAMETERS AND PLATELET AGGREGATION - A POSSIBLE ASSOCIATION WITH METEOROLOGICAL FACTORS?

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In our present study we investigated seasonal patterns in hemorheological parameters and platelet aggregability and their potential association with meteorological factors. ADP, collagen and epinephrine induced platelet aggregation was assessed in 2694 vascular patients treated with 100 mg acetylsalicylic acid according to the method of Born. In subgroups of patients' hematocrit, fibrinogen, red blood cell aggregation as well as plasma and whole blood viscosity were also simultaneously measured. Data were divided into 12 groups according to the months of the year based on the time-point of the examination. External temperature at 8 a.m., air pressure at 8, 9 and 10 a.m., daily minimal and maximal temperatures, relative humidity and the daily amount of precipitation were measured in the local station of the Hungarian Meteorological Service. Significantly higher fibrinogen levels were found in the winter months ($p<0.01$). Red blood cell aggregability proved to be significantly higher in the winter than in the summer months ($p<0.0001$). A clinically minor elevation was also observed in hematocrit levels in winter ($p<0.01$). Epinephrine-induced platelet aggregation was highest in April and May, while its minimum was in August and September ($p<0.05$). The investigated meteorological factors did not seem to affect these seasonal differences directly.

S25A
MICROVASCULAR BED SPECIFIC REGULATION OF WALL SHEAR STRESS
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Regulation of wall shear stress (WSS) in microvessels, responsible for the large portion of peripheral resistance is thought to provide optimal power loss in the circulation. In arterioles, increases in WSS elicited by increases in flow or fluid viscosity elicit substantial, endothelium dependent dilations, mediated by nitric oxide (NO), dilator prostaglandins (PGs) and reactive oxygen species. In venules, increases in WSS result in dilations, which however, are significantly tempered by the release of constrictor prostaglandins (cPGs) in addition to NO and dilator PGs. It is known that WSS is the function of wall shear rate (WSR) and blood viscosity (hematocrit and plasma viscosity), which however, can be substantially different in arterioles and venules. Thus, it is likely that regulation of WSS is achieved by different mechanisms in arterioles and venules, dependent on the values of WSR and apparent viscosity. Interestingly, in cerebral arterial vessels increases in flow elicit constrictions. Because the brain is closed in the cranium, even small increase in volume would greatly increase intracranial pressure, thus in this case the Monroe-Kelly law seems to prevail. Systemic increases in WSS via increases in hematocrit elicit reduction in blood pressure, suggesting that the overall aim of WSS regulation is to optimalize energy dissipation in the circulatory system, however, complex interrelationship exist among the rheological parameters, structural and functional properties of microvascular networks and specific needs of organs and tissues. Grants: AHA NE Aff. 0555897T; Hungarian Sci. Res. Funds/OTKA-T48376, K67984; Health Sci. Council/ETT 364/2006.

S25B
WALL SHEAR STRESS DISTRIBUTION IN THE ARTERIAL SYSTEM.
RECONSIDERATIONS BASED UPON IN VIVO MEASUREMENTS
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Based upon theory, wall shear stress (WSS), an important determinant of endothelial function and gene expression, is considered to be constant along the arterial tree and similar in a particular artery across species. *In vivo* measurements, however, avoiding theoretical assumptions, show that these considerations are far from valid: in humans mean WSS is higher in the carotid artery (1.1-1.3 Pa) than in the brachial (0.4- 0.5 Pa) and femoral (0.3-0.5 Pa) arteries. Also in animals mean WSS varies along the arterial tree. In arterioles mean WSS varies between 1.0 and 5.0 Pa and is dependent on the site of measurement. Across species mean WSS in a particular artery decreases linearly with body mass, in the descending aorta from 8.8 Pa in mice to 0.5 Pa in humans (flow velocities being similar). The observation that mean WSS is far from constant along the arterial tree indicates that Murray's cube law on flow-diameter relations cannot be applied to the whole arterial system. These findings also imply that in *in vitro* investigations no average shear stress value can be taken to study cellular adhesion and the effects on endothelial cells derived from different vascular areas or from the same artery in different species.

S25C
WALL SHEAR STRESS AND THE GLYCOCALYX
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The endothelial glycocalyx is positioned between flowing blood and the vascular wall. Recent studies demonstrate that an intact glycocalyx is essential for fluid shear stress sensing and subsequent biochemical endothelial activation. In vitro studies on cultured endothelium show that upon enzymatic removal of glycocalyx glycosaminoglycans, the endothelial cells lose their ability to adapt to flow profiles within 24 hours, i.e., no effect of shear stress on cell alignment and migration behavior. At the same time, exposure of fluid shear stress to the endothelial surface triggers glycocalyx synthesis. In addition, experimental studies reveal that thinner glycocalyces are present at specific atherogenic sites of arterial bifurcations predisposed to develop atherosclerotic lesions. These findings are consistent with the hypothesis that modified glycocalyx synthesis and breakdown dynamics at atherogenic risk areas exposed to complex flow profiles contributes to the increased vulnerability of these sites to systemic atherogenic risk factors. It is therefore to be expected that systemic glycocalyx modification by atherogenc risk factors or therapeutic approaches is related to cardiovascular vulnerability. Based on these findings and hypotheses, several clinical studies are ongoing to test whether monitoring of (microvascular) glycocalyx dimensions can be used as a diagnostic tool to identify early atherogenic vulnerability.

S25D
WALL SHEAR STRESS AND ENDOTHELIAL CELL GENE EXPRESSION

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Shear stress is the frictional force that the flow of blood exerts at the endothelial surface of the vessel wall. This physical force is registered by the endothelial cell and translated into the activation of specific signal transduction cascades within cell-cell contacts, caveolae and focal adhesion contacts, many of which result in the activation of transcription factors and altered gene expression. The effects observed are marked as following cell isolation and culture under static conditions, endothelial cells take on a more "inflammatory" phenotype characterised by low nitric oxide production, elevated adhesion molecule and angiotensin-converting enzyme expression as well as endothelin production. Exposing endothelial cells to levels of constant shear stress generally classed as "venous" actually does little to alter this expression profile while "arterial" levels of shear stress revert the endothelial cell phenotype to that seen in situ. However, constant shear stress is a relatively artificial phenomenon and generally not observed in vessels in situ and disturbed blood flow plays an important role in the development of atherosclerosis, particularly in anatomical regions where flow conditions are low and/or oscillatory. The signalling cascades that regulated gene expression under constant and oscillatory shear stress will be discussed.

Thursday, August 28th
Angiotensin II type 2 receptor in the control of vascular resistance in vascular and metabolic diseases
Parallel Symposium – S26

S26A
INVOLVEMENT OF ANG II IN ISCHEMIA-INDUCED ANGIOGENESIS
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A good blood supply to tissues is essential for adequate function and numerous pro- and antiangiogenic factors regulate this complex process.
Angiotensin II plays an important role particularly in neo-vascularization of ischemic tissues. The respective role of the AT_1 and AT_2 receptors however remains controversial.
Ang II binding to the AT_1 receptor stimulates the hypoxia-inducible factor HIP-1 through PKC and NADPH oxidase and transactivates the VEGF receptor that stimulates PI3K and Akt leading to eNOS activation, inhibition of apoptosis and angiogenesis. AT_1 receptor stimulation also transactivates the EGF receptor causing angiopoietin and MMP stimulation. This proangiogenic effect is prevented by AT_1 receptor blockade or VEGF antibodies and is altered in eNOS-deficient mice as well as in tissues of AT_1 knock-out animals. AT_1 receptor also stimulates the expression of pro-inflammatory molecules through NF□B activation that affect the neovascularization process.
AT_2 receptor stimulation appears to oppose the effect of the AT_1 receptor causing vasodilation and having anti-growth properties. Through activation of various phosphatases, the AT_2 receptor reduces the growth promoting effect of the AT_1 receptor. It stimulates caspase while reducing phosphorylation of the anti-apoptotic Bcl2 leading to apoptosis and inhibition of neovessel formation. AT_2 receptor blockade enhances angiogenesis in rat subcutaneous sponge granuloma and vessel growth is significantly increases in the ischemic hind-limb in AT_2 deleted mice. AT_2 stimulation is also able to raise BK and NOS activity leading to PKG activation and angiogenesis. Such stimulatory pathway was observed in culture conditions with severe hypoxia and in angiotensinogen overexpressing mice. This effect was impaired in AT_2 deficient mice.
In conclusion, Ang II exerts a double-edge role in angiogenesis signaling where the AT_2 receptor appears usually to counteract the effect of the AT_1. The balance of AT_1/AT_2 expression as well as the vessel size, age, hypertension and the pathophysiological environment may affect the role of the RAS and of its blockade.

S26B
FUNCTIONAL ROLE OF AT2 RECEPTORS IN CARDIOVASCULAR DISEASE: EVIDENCE FROM IN VIVO STUDIES
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The octapeptide angiotensin II is well recognised as the major mediator of the renin-angiotensin system, causing a range of AT_1 receptor-mediated effects including acute cardiovascular effects (e.g. vasoconstriction) as well as longer-term trophic effects (e.g. cardiac and vascular remodelling). On the other hand, there is much evidence that supports the view that angiotensin II (Ang II), acting on AT_2 receptors, causes inhibitory effects (e.g. vasorelaxation, antigrowth) that counterbalance the excitatory AT_1 receptor-mediated actions of Ang II. Such functional interactions may play a role in the beneficial effects of AT_1 receptor antagonists (sartans). In this context, experimental data will be presented that assesses the relative contribution of AT_2 receptors to sartan-induced haemodynamic and structural remodelling effects. In addition, there are a number of other angiotensin peptide fragments such as Ang-(1-7) and Ang III that exert acute and chronic cardiovascular effects, some of which may be due to stimulation of AT_2 receptors or other non-AT_1 receptors. Thus, the potential for angiotensin peptides to exert acute cardiovascular effects, as well as counter-regulatory (antigrowth) effects opposing Ang II on cardiac remodelling, will also be explored. Collectively, from *in vivo* experiments, AT_2 receptors are likely to limit AT_1 receptor-mediated overactivity in cardiovascular disease.

S26C
MOLECULAR MECHANISM RESPONSIBLE FOR ANGIOTENSIN II TYPE 2 RECEPTOR-INDUCED RHOA INACTIVATION AND VASODILATION
C Guilluy [1], M Rolli-Derkinderen [1], L Loufrani [2], A Bourgé [1], D Henrion [2], L Sabourin [3], G Loirand [1], P Pacaud [1]
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The small G protein Rho signaling pathways are recognized as major regulators of cardiovascular functions, and activation of Rho proteins appears to be a common component for the pathogenesis of hypertension and vascular proliferative disorders. Recent evidence suggests that modulation of Rho protein signaling by phosphorylation of Rho proteins provides an additional simple mechanism for coordinating Rho protein functions. Phosphorylation of RhoA by cAMP- or cGMP-activated kinase on Ser 188 induces cytosolic sequestration of RhoA through increased interaction with guanine dissociation inhibitor, thereby resulting in inhibition of RhoA-dependent functions. Here we show that stimulation of angiotensin II (Ang II)

type 2 receptor (AT2R) in vascular smooth muscle cells induces Ser 188 phosphorylation of RhoA independently of cAMP- or cGMP-activated kinase. We identify the Ser/Thr kinase Ste20-related kinase SLK as a new kinase phosphorylating RhoA on Ser 188. Activation of the signaling cascade involving Src homology 2 domain-containing protein-tyrosine phosphatase 1, casein kinase II and SLK is responsible for RhoA phosphorylation and inhibition of RhoA-mediated arterial contraction induced by AT2R activation. These results thus identify the molecular mechanism linking AT2R to RhoA inhibition and vasodilation.

S26D

ANGIOTENSIN II TYPE 2 RECEPTOR-DEPENDENT DILATION IS REDUCED IN TYPE II DIABETES DUE TO COX-2 GENERATED THROMBOXANE A2 AND REACTIVE OXYGEN SPECIES.

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Angiotensin II controls local blood flow through type 1 (AT1R) and 2 (AT2R) receptors. AT2R-stimulation induces vasodilatation, although in hypertension, aging or diabetes, it induces weak vasodilatations or vasoconstrictions. We investigated AT2R stimulation in RA from type 2 diabetic rats. Mesenteric arteries were isolated from Zucker diabetic fatty (ZDF) and lean Zucker (LZ) rats treated with the antioxidant tempol or water. Arterial function was studied using in vitro myography. In ZDF rats AT2R-induced dilation, reduced by half, was NO-independent, compared to LZ rats. AT2R and eNOS expression levels were similar in the two strains. AT2R-dilation in ZDF rats was equally restored to control level after acute ROS reduction (tempol), cycloxygenase-2 (NS398) or Thromboxane A2 receptor (SQ29548) inhibition. Cycloxygenase-2 expression and ROS production were significantly elevated in ZDF rats' arteries. After chronic tempol AT2R-dependent dilation in ZDF rats was equivalent to that in LZ rats and involved both NO and cycloxygenase-2 derivatives. ROS production was reduced but COX-2 still present in tempol-treated ZDF rats. These findings might be important to consider in the choice of vasoactive drugs in diabetic patients. Treatments with antioxidant properties might be preferred.

Thursday, August 28th
Hot issues in the cerebral microcirculation I.
Parallel Symposium – S27

S27A

MITOCHONDRIAL INFLUENCES ON CEREBRAL VASCULAR TONE IN HEALTH AND DISEASE

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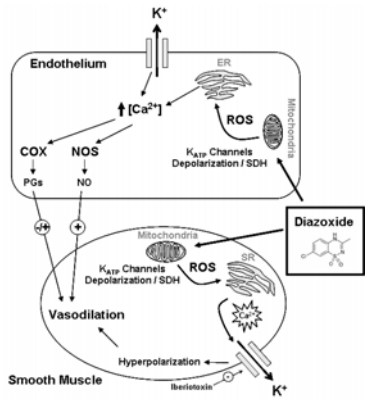
Mitochondria affect cerebral vascular tone by mechanisms involving activation of mitochondrial ATP-sensitive potassium channels (mitoK_{ATP}) and the generation of reactive oxygen species (ROS). We explored the effects of diazoxide, a mitoK_{ATP} activator, on cerebral arteries of Zucker obese (ZO) rats with insulin resistance (IR) (a metabolic disorder preceding type 2 diabetes) and their lean (ZL) controls. Diameter measurements showed diazoxide-induced cerebral artery vasodilation was diminished in ZO compared to ZL rats. Iberiotoxin and a ROS scavenger reduced responses to diazoxide in both groups implicating calcium-activated potassium channels and ROS. Diazoxide-induced vasodilation was reduced by endothelial denudation or nitric oxide synthase inhibition. In contrast, indomethacin potentiated vasodilation in ZL rats implicating vasoconstrictor prostanoids but ZO rats were unaffected. Fluorescence microscopy showed decreased mitochondrial depolarization and superoxide production in response to diazoxide in ZO compared to ZL arteries. Thus, cerebrovascular dilation to mitochondrial-derived factors involves the integration of endothelial and smooth muscle mechanisms. Furthermore, IR impaired cerebral vasodilation in ZO rats by diminishing the mitochondrial response to diazoxide. These results imply that patients suffering from the metabolic syndrome may exhibit cerebral vascular function and inadequate blood flow responses due to impaired mitochondrial-dependent vasodilation. Supported by HL-030260, HL-065380, and HL-077731 from NIH.



S27B

ANGIOGENIC POTENTIAL OF VEGF AND NEURAL STEM CELLS IN THE BRAIN

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2 aspects of VEGF as a major player in angiogenesis were investigated.

1. Angiogenic potential of adult neural stem cells in culture: These cells showed a hitherto unknown angiogenic potential: VEGF is expressed and a regional diversity in the expression pattern of VEGF receptors exists in the three types of adult neural stem cells. The expression pattern can be changed by exogenous VEGF.
2. Potential of VEGF to induce new capillaries in the intact brain.

First approach: Injection of adenoviruses into rat brains to overexpress VEGF165 resulted in two functionally different patterns of newly formed vessels: high density of moderate- sized vessels resulting in an increased local cerebral blood flow and a moderately increased density of large vessels resulting in a decrease in local cerebral blood flow.

Second approach: Transgenic mice having a brain specific overexpression of VEGF165 showed, despite a drastical increase in capillary density in white matter structures (70-185%), no difference in normocapnic local cerebral blood flow and only moderate increase of hypercapnic blood flow in some structures compared to nontransgenic mice.

S27C

REMODELING OF RAT PIAL MICROCIRCULATION AFTER TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION

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The aim of this study was to assess the remodelling of pial arteriolar networks in the penumbra area during different time intervals of reperfusion in rats subjected to the transient middle cerebral artery occlusion (MCAo). In vivo pial microcirculation was observed by a fluorescence microscopy through a cranial window. MCAo was performed by nano-filament insertion for 2 hours. After 1, 7, 15 and 30 days of reperfusion the arteriolar networks were mapped by stop-frame images; arterioles were classified by Strahler's ordering scheme and their diameters and lengths were measured with a computer-assisted method. At the end of the different periods of reperfusion, the penumbra area presented order 1, 2 and 3

pial arterioles organized in anastomotic branchings overlapping the territory of the occluded arteries ensuring blood supply. After 30 days of reperfusion, the anastomoses were increased in number and geometrically rearranged in the same way as observed under baseline conditions. To evaluate the remodelling of the pial microvascular networks, symmetry or asymmetry of new branchings was quantitatively defined by the segments/elements ratio that regained the baseline values after 30 days of reperfusion. In conclusion, after MCAo the arteriolar networks regained the same pattern of bifurcation as observed under baseline conditions.

S27D

MICROVASCULAR CHANGES IN CEREBRAL CORTEX AFTER SUBARACHNOID HEMORRHAGE IN THE RAT

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This study aimed to assess functional changes in the microcirculation after subarachnoid hemorrhage (SAH) in the model in which only moderate narrowing of the lumen of large extraparenchymal arteries is observed. The experiments were performed in adult, male Sprague-Dawley rats. SAH was induced by the puncture of the artery at the circle of Willis. Microflow in cerebral cortex was measured bilaterally up to 40 minutes after SAH (acute phase) and 3, 24, 48 and 72 hours after the bleeding, using laser Doppler flowmeter. Following SAH ipsilateral microflow did not respond to the tested vasodilators (acetylcholine, carbon dioxide, papaverine) up to 48 hours. At 72 hours after SAH the recovery of the response to carbon dioxide and papaverine was noted only in animals with moderate ischemia in the acute phase (microflow below 30% of control for 15-20 min). In all cases with severe ischemia (microflow in acute phase below 30% of control for more than 40 min) no recovery was observed. In addition, in the latter group, at 72 hours after the bleeding cerebrovascular resistance decreased after elimination of vasoconstrictor eicosanoids. Our results suggest that delayed microcirculatory dysfunction may develop after SAH independent of the late vasospasm of large arteries.

S27E

SIMULTANEOUS IMAGING OF CHANGES IN CEREBRAL BLOOD FLOW AND TISSUE pH DURING K^+-INDUCED CORTICAL SPREADING DEPRESSION

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Spreading depression (SD) are propagating waves of cellular depolarization that are associated with classical migraine and acute brain lesions. A multimodal imaging strategy was developed to visualize SD-associated cerebral blood flow (CBF) changes, and the corresponding intracellular pH shifts indicative of ionic and metabolic responses.
Within a cranial window prepared in halothane-anesthetized rats, repeated SD was elicited by topical application of high-K^+ medium. Two separate CCD cameras, synchronized to suitable illumination, were used for the dual imaging of changes in CBF (laser speckle contrast imaging) and Neutral red fluorescence (NR; a pH-sensitive vital dye, administered i.p.).
Laser speckle SD signatures displayed features similar to laser Doppler flowmetry data obtained previously; i.e. an initial, short CBF drop, preceding a marked hyperemia, followed by a long-lasting hypoperfusion. SD-related NR fluorescence reflected a biphasic intracellular acidification: i.e. an initial, sharp peak that coincided with hyperemia, followed by a longer-lasting acidification. The latter phase developed 80 to120 sec after the hyperemia peak.
We propose that the initial intracellular acidification corresponds to SD-related, non-specific disruption of trans-membrane ionic gradients. In contrast, the long-lasting acidification may reflect an imbalance between oxygen demand and oxygen blood supply. This study was supported by a grant from the Wellcome Trust (079430/A/06/Z).

Thursday, August 28th
Lymphatic functional biology: new insights II.
Parallel Symposium – S28

S28A
LYMPHEDEMA THERAPEUTICS: THE POTENTIAL FOR PHARMACOTHERAPY
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Lymphedema is the clinically relevant pathological disorder that results from chronic lymphatic vascular insufficiency. The disease is prevalent, progressive and often under-recognized. Current therapeutic options are quite limited, and are generally restricted to physical maneuvers designed to transiently mobilize tissue fluid and to compress the tissues to effect a reduction in volume.
Recent efforts at delineating the pathophysiology of lymphedema have increasingly relied upon small animal model systems that also permit characterization of the molecular responses to lymphatic vascular insufficiency. Such studies suggest that pharmacological and other molecular approaches may afford the opportunity to reduce edema volume, reverse pathological changes in tissue architecture, and improve circulatory and immune function in this disease. Relevant animal models and derived experimental data will be discussed.

S28B
THE ROLE OF LYMPHATICS IN METASTASIS OF TUMOURS
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Melanoma metastasizes primarily via gaining access to local lymphatic capillaries in the region that the tumours originally arise. The mechanisms through which melanoma cells locate, identify and invade the lymphatic capillaries are now being elucidated. Three possible mechanisms for lymphatic invasion are passive migration/flow, lymphangiogenesis, or metastatic chemotaxis. The former hypothesis requires no specific interaction between the tumour and the lymphatics, whereas the latter two would predict exactly such an interaction. The finding that molecules that drive either directed metastatic chemotaxis (e.g. CCL21), or lymphangiogenesis (e.g. VEGF-C) can increase metastasis indicate that these two mechanisms contribute towards lymphatic invasion and spread. We have identified a mechanism whereby tumour cells can mediate their own migration by upregulation of chemoreceptors for secreted lymphatic chemokines. The interaction between lymphatic secreted chemokines and tumour expressed chemokine receptors in human and murine melanomas will be demonstrated.

S28C
FLUID DYNAMICS IN THE INITIAL LYMPHATIC SYSTEM
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Fluid kinetic in initial diaphragmatic lymphatics was studied in anesthetized, paralyzed and mechanically ventilated rats. Two types of submesothelial lymphatic lacunae were distinguished in the muscular and in the tendineous medial regions of the diaphragm: complex loops and linear vessels. Lymph vessel diameter was significantly greater ($p<0.001$) in linear vessels (103.4 8.5 microns; n=18) than in loops (54.6 +/- 3.3 microns; n=21). Conversely, flow velocity, obtained from intraluminar speed of a bolus of fluorescent dextrans injected in the vessel, was lower ($p<0.001$) in linear vessels (26.3 +/- 1.4 microns/sec) than in loops (51.3 +/- 3.2 microns/sec). Lymph flow was similar in linear vessels and in individual vessels of complex loops, averaging 1.6 +/- 0.3 nl/min. Flow direction was centripetal in linear vessels, more complex and evidently controlled by intraluminar unidirectional valves in loops. The results suggest that loops might be the preferential site of lymph formation, while linear vessels would be mainly involved in centripetal propulsion of newly formed lymph towards collecting diaphragmatic ducts. Hence, optimisation of diaphragmatic lymphatic flow throughout the respiratory cycle would be achieved through a certain degree of specialization even within the same hierarchic order of submesothelial lacunae.

S28D
AUTOLOGOUS CHEMOTAXIS DRIVES DENDRITIC CELL TRAFFICKING TO LYMPHATICS
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The lymphatic system is the primary route for immune trafficking to the lymph node (LN). Dendritic cells (DC) express CCR7 upon maturity and CCR7 signals are implicated in DC trafficking via lymphatics. It remains unclear, however, how peripheral DCs find lymphatic vessels and mobilize to LN, or how the biophysical environment influences this. We demonstrated that tumor cells express both CCR7 and its ligands, and use these signals with interstitial flow to direct themselves to draining lymphatics via autologous chemotaxis. Therefore, we investigated whether DCs also utilize CCR7-

dependent autologous chemotaxis. Adoptive transfer of DCs from wild type mice (WT, CCL21/19 secreting), mice lacking CCL19 and CCL21 (Plt), and mice lacking CCL19 (ELC-/-) demonstrated that the absence of DC-derived CCL21 or CCL19 reduced homing to the draining LN, even when lymphatic signals were intact. 3D invasion assays demonstrated that DCs from all backgrounds responded to lymphatic signals, confirming CCR7 functionality. However, addition of slow flow only enhanced migration of WT cells. DCs from either plt or ELC-/- mice failed to respond to flow. These data indicate that CCR7-mediated autologous chemotaxis is required for DC mobilization via lymphatics, and explains how increased lymph drainage during inflammation assists recruitment of DCs to LN.

Thursday, August 28th
The morphology and function of the two distinct portions of the renal afferent arteriole
Parallel Symposium – S29

S29A
FLIUD FILTRATION THROUGH NANO-CHANNELS OF THE AFFERENT ARTERIOLE (AA)
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Utilizing multiphoton laser (*in vivo*) and atomic force (*in vitro*) microscopies, we studied the morphology and function/permeability of endothelial nano-channels in AA of rats and mice. Part of plasma in AA does not enter glomerulus, rather filtrated into the interstitium via endothelial nano-channels existing at the renin positive portion of AA. The fluorescent tracer of filtration was present in mesangial interstitium and macula densa tubular lumen. In isolated perfused JGA, obstruction of proximal tubule caused a significant reversible volume increase in Bowman space, JGA interstitium, macula densa cells and the pertaining tubular lumen. These data suggest fluid movement from AA into JGA and the distal tubule. The endothelial nano-channels (diameter: 50-200nm, AFM, HUVEC) exhibit round openings with gyrus-like upfolding and a cone-shape channel. The number of channels was increased by VEGF treatment. This effect was eliminated by p38 inhibitor SB203580. We described a new morphology of the renin positive portion of AA with endothelial nano-channels of functional importance, namely feed-forward glomerular microcirculatory regulating mechanism.

S29B
MULTIPHOTON IMAGING OF THE JUXTAGLOMERULAR APPARATUS
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The juxtaglomerular apparatus (JGA) represents a major structural component of the renin-angiotensin system, and is one of the most important regulatory sites of renal salt & water conservation and blood pressure maintenance. The process of glomerular filtrate formation and regulation of renal hemodynamics, including the tubuloglomerular feedback (TGF) mechanism from the macula densa and renin release, involves the complex interaction of a number of different cell types in the JGA. We applied multiphoton excitation fluorescence imaging, a state-of-the-art confocal imaging technique ideal for deep optical sectioning of living tissues. Using the intact kidney and JGA we directly visualized JGA functions including the extracellular ATP-mediated calcium wave of TGF, dynamics and regulation of JGA renin granular content by a variety of stimuli, renin release and local activity, and the significant fluid flow in the JGA interstitium. Also, we performed quantitative multi-photon imaging of basic functions in renal (patho)physiology including glomerular filtration and permeability of the glomerular filtration barrier, local blood flow and tubular flow, concentration, dilution, as well as more integrated and complex functions like TGF-mediated oscillations in glomerular filtration and tubular flow rate. New visual data challenge a number of existing paradigms in renal (patho)physiology.

S29C
CONNEXIN PATTERN OF MOUSE RENAL AFFERENT ARTERIOLES
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The different cell types at the terminal parts of the renal afferent arterioles including smooth muscle, endothelial, renin producing and mesangial cells form gap junctions among each other, underlining the relevance of intercellular communication in this specific region. Since gap junctions are made up from connexin proteins, we have analyzed the connexin expression in the terminal parts of afferent arterioles focussing on vascular connexins such as Cx37, 40, 43 and 45.
We found Cx37 mainly in the endothelium and to a lesser extent in intraglomerular cells near the vascular pole and in renin expressing cells. Cx40 was also found in the endothelium, but more prominently in renin expressing cells as well as in intra- and extraglomerular mesangial cells. Cx43 was seen in the endothelium, renin producing cells and mesangial cells. Cx45 was restricted to the vascular smooth muscle cells. Amongst these connexins, Cx40 appears to play a major role since its genetic deletion also reduces Cx37 and C43 expression and even leads to disappearance of Cx37 and Cx43 from renin expressing cells.
Since deletion of Cx40 also causes a major disturbance of renin expression and renin secretion, gap junctions formed by Cx40 appear of critical relevance for the function of the juxtaglomerular apparatus.

S29D
PERMEABILITY TO WATER IS INCREASED IN GLOMERULAR AND SYSTEMIC MICROVESSELS OF MALE MUNICH WISTAR FROMTER RATS.

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Cardiovascular disease is the leading cause of death in the western world. Recently albuminuria has been shown to predict cardiovascular mortality and has been proposed to reflect systemic endothelial dysfunction. (Yuyun Int J Epidemiol 2004; 33:189-198). The Munich Wistar Fromter (MWF) rat strain develops spontaneous albuminuria and therefore is an interesting experimental model to study alterations of endothelial function under conditions of increased albuminuria. Urine from male MWF rats (15-24 wks) was collected using metabolic cages and the protein:creatinine ratio measured. (MWF:15.5±1.67 n=14 Wistar:1.65±0.22 n=6, p<0.0001). Hydraulic conductivity (L_p) was measured in individually perfused microvessels of the mesentery. Male MWF rats had significantly increased L_p (cm $s^{-1}cmH_20^{-1}$) compared with age matched Wistar controls. (MWF: $6.39\pm1.75\times10^{-7}$ n=7; Wistar: $2.27\pm0.44\times10^{-7}$ n=8, p=0.02). Glomerular ultrafiltration co-efficient (L_pA/V_i) was measured using an oncometric assay. (Salmon J Physiol 2006; 570(1):141-56). The increase in systemic L_p was accompanied with an increase in glomerular L_pA/V_i. (MWF: 1.27±0.12 $min^{-1}mmHg^{-1}$ n=9; Wistar: 0.81±0.14 $min^{-1}mmHg^{-1}$ n=3, p=0.065). These results suggest the link between glomerular albuminuria and systemic cardiovascular disease is accompanied by similar defects in the barrier function of the microvessel wall. Supported by BHF.

S30A

PERIODIC DISAPPEARANCE OF RBC FROM FOCAL CAPILLARY NETWORK DURING K+-INDUCED CORTICAL SPREADING DEPRESSION IN RODENTS

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Mechanisms controlling RBC redistribution and velocity in a capillary network under constant perfusion pressure are not known. Recently we developed an automated method for tracking vast numbers of FITC-labeled RBCs in microvessels using a high-speed confocal microscope system with Matlab-domain image processing software KEIO-IS2 (Microcirculation (2008) 15:163-174). To examine the effect of neuronal deactivation on capillary RBC flow, we used 20 rats in which we carried out in vivo staining of single capillaries with FITC-dextran and of astrocytes with sulforhodamine 101. During video-recording of RBC flow, concentrated K+ was microinjected into the cortex to induce cortical spreading depression (CSD), during which neurons are transiently completely depolarized. We found periodic exclusion of RBC in association with wave-ring spread of CSD, evidenced by negative deflections in DC potential and increased background brightness. No detectable diametric changes of the pertinent capillaries were observed. The momentary RBC exclusion was followed by increases in RBC flow and RBC attendance. Endfeet of astrocytes, which were stained brown color, were seen to cover microvessels and changed their morphology slowly, but no direct correlation to wave-ring spread was observed during CSD. The mechanism of the RBC exclusion seemed to be related to RBC flow resistance in capillaries.

S30B

tPA IN CEREBRAL ISCHEMIA: DELIERY. SIGNALING, AND OUTCOME

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Babies experience hypoxia (H) and ischemia (I) from stroke or problems with delivery or post delivery respiratory management. The only approved stroke treatment is tissue plasminogen activator (tPA). However, H/I impairs pial artery dilation (PAD) to hypercapnia and hypotension in piglets, exogenous tPA potentiates this effect and blockade of endogenous tPA-mediated vasoactivity prevents it. Mitogen activated protein kinase (MAPK), a family of at least 3 kinases, ERK, p38 and JNK, is upregulated after I. Coupling of tPA to RBCs may reduce its CNS toxicity through spatial constraints. In this study, hypercapnic PAD was blunted after H/I, reversed to vasoconstriction in pigs treated with tPA, but dilation was maintained at levels nearly equivalent to pre-H/I values in animals given RBC-tPA. CSF and brain parenchymal ERK MAPK was elevated by H/I, insult upregulation was further potentiated by tPA, but blunted by RBC-tPA. U 0126, an ERK MAPK antagonist, helped maintain PAD. Increased numbers of degenerating neurons in the CA1 hippocampus and parietal cortex after H/I was blunted by U 0126, but potentiated by tPA. These data suggest that RBC coupling to tPA may offer a novel approach towards increasing the benefit/risk ratio of thrombolytic therapy for CNS disorders associated with H/I.

S30C

EFFECTS OF ISCHEMIA REPERFUSION INJURY ON GENE EXPRESSION OF THE ENDOTHELIN SYSTEM IN CEREBROARTERIAL ENDOTHELIAL CELLS

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Objective: Transient focal brain ischemia in rats has been described to result in a *de novo* gene expression of the endothelin (ET)B receptor in the smooth muscle cells mediating contraction. In addition, there is an enhanced relaxation of the isolated middle cerebral artery (MCA) due to stimulation of ET(B) receptors located on the endothelium. The aim of the present study was to determine whether this enhanced relaxation is due to an upregulation of the ET(B) receptor-mRNA in the endothelial cells.
Methods: Adult male Sprague-Dawley rats underwent a 2 h MCA occlusion using the intraluminal filament technique followed by reperfusion. After sacrifice 2 days later, the brain was removed and the previously occluded MCA selectively perfused with an ice-cold buffer to disrupt the cell membranes. The total RNA was isolated from the perfusate and underwent reverse transcription followed by quantitative polymerase chain reaction (qPCR) analysis of the mRNA for ET(B), ET(A), ET-1, and endothelin converting enzyme (ECE) with elongation factor (EF)-1 serving as the house-keeping gene.
Results: After ischemia the total mRNA content was threefold higher than in control vessels ($p<0.05$). The slopes of the dilution curves did not differ between the individual primers indicating similar efficiency of amplification. The expression of the ET(A) mRNA was not detectable under control conditions or after ischemia. The expression of ET(B) mRNA was increased after ischemia with the level of significance reaching 0.03 and 0.07 at 24 and 48 hours respectively. Even more pronounced were the upregulation of ET-1 and ECE mRNA expression after 24 and 48 hours.
Conclusion: The results indicate marked alterations in the cerebroarterial endothelium gene expression after ischemia reperfusion injury. We found significantly increased ECE, ET-1, and ET(B) mRNA expression thus further emphasizing the importance of the vascular ET-system in the pathophysiology of postischemic events.

S30D

ACTIVE CONSTRICTION OF BRAIN CAPILLARIES IN VITRO AND IN VIVO

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The role of capillaries as active elements of the regulation of blood flow has long been a controversial issue. The contractile properties of central nervous system pericytes in culture are well documented, and more recently the ability of capillaries in organotypic preparations of the retina and cerebellum to react to pharmacological or electrical stimuli has been reported. The study of capillary vasoactivity in the brain *in vivo* has been hampered by the difficulty to image these vessels in the depth of the tissue. We have used two-photon microscopy to image dynamic changes of capillary morphology upon stimulation with the thromboxane-A2 analogue U46619, both in cortical slices and in the intact, blood-perfused brain. In slices of the parietal sensory cortex, we observed significant constrictions after superfusion with U46619. Observed through a closed cranial window in the brain cortex of living, anesthetized mice, the topical application of U46619 also elicited localized reductions of capillary diameters, which were paralleled by changes in red blood cell velocity and perfusion rates. Taken together, these results suggest that capillaries may actively change cortical cerebral blood flow, challenging the concept of arterioles as the sole site of cerebral blood flow regulation.

S30E

MEASUREMENT OF BLOOD-BRAIN BARRIER PERMEABILITY AND STEROID EFFECT IN TUMOROUS EDEMA FORMATION BY MEANS OF MR IMAGING

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Administration of steroids in peritumoral brain edema is an important treatment in neurooncology. Both the edema development and the steroid effect are in strong correlation with blood-brain barrier (BBB) permeability. Tracking the water content of the edema and the integrity of the BBB can strongly support diagnostic and monitoring efforts.

We present six cases with brain edema, in which water content of the edema was measured with water maps. These maps are calculated from voxelwise measured T1 values. The effect of dexametheson was followed for 72 hours. The water content of the edema is significantly decreased (from 83.72% to 82.9%: $p>0.001$) due to the applied steroid treatment.

Preliminary results in a single case are also presented in which the BBB integrity was evaluated by first-pass T2-weighted susceptibility measurement. The first-pass susceptibility effect of gadolinium bolus is tracked in the tumor 45-60 seconds after the administration of the contrast agent. The following BBB parameters were calculated: permeability (K_t), fractional plasma volume (v_p) – from which the relative Cerebral Blood Volume (rCBV) can be easily calculated – and fractional extracellular volume (v_e).

Friday, August 29th
Role of nitrite in cardiovascular homeostasis
Parallel Symposium – S31

S31A
NITRITE AND ISCHEMIA-REPERFUSION INJURY
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Background: Nitrite has long been considered an inert oxidative metabolite formed following enzymatic synthesis of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS). Nitrite represents a stable NO storage form that accumulates in the circulation and in tissues throughout the body. Total circulating and tissue nitrite levels are also influenced by dietary intake of nitrate and nitrite. Recent experimental evidence suggests that nitrite is reduced to NO during ischemic or hypoxic events via a number of enzymatic reactions (i.e., hemoglobin, myoglobin) or direct disproportionation (i.e., acid reduction). We hypothesized that administration of nitrite would increase NO bioavailability in the myocardium during ischemia/reperfusion (I/R) and thereby protect the heart. Therefore, we investigated the effects of acute nitrite administration (41.25 to 165 µg/kg, i.c.) as well as dietary nitrite supplementation on the response to myocardial ischemia- I/R injury in an *in vivo* murine model system. Myocardial infarct size per area-at-risk was reduced by 67% ($p < 0.01$ vs. Vehicle) with bolus intracardiac nitrite therapy (165 µg/kg) following 30 minutes of left coronary artery ischemia and 24 hours of reperfusion. Post-ischemic left ventricular function was also significantly preserved in nitrite treated mice at 24 hours following reperfusion. We also examined the effects of nitrite administration (165 µg/kg, i.p.) at 24 hours prior to myocardial I/R injury to pharmacologically precondition the myocardium. Myocardial infarct size per area-at-risk was reduced from 53 ± 5% to 18 ± 4% ($p < 0.01$ vs. Vehicle) following nitrite preconditioning. Recently, we have examined the effects of oral nitrite administration (50 mg/Liter for 7 days) in the drinking water on total body nitrite/nitrate, NO-heme, and nitroso levels as well as the severity of myocardial I/R injury. We have clearly demonstrated significant increases in both plasma and myocardial tissue nitrite and NO-metabolite storage. Our study also demonstrates that myocardial tissue nitrite is converted to NO during ischemia and that myocardial infarct size is reduced by 48% ($p < 0.05$ vs. standard diet) following I/R injury. Interestingly, mice fed a nitrite deficient diet exhibited significant reductions in both plasma and myocardial nitrite and NO-metabolite levels and experienced a 59% increase in myocardial infarct size following myocardial I/R. These data clearly demonstrate the critical importance of tissue nitrite stores in the setting of I/R injury and provide evidence that administration of nitrite either via acute injection or via dietary manipulations significantly ameliorates cardiac I/R injury.

S31B
NITRITE MODULATES MITOCHONDRIAL FUNCTION TO MEDIATE ACUTE AND DELAYED CYTOPROTECTION AFTER I/R
S Shiva

Recent studies have shown that physiological concentrations of nitrite mediate cytoprotection after ischemia/reperfusion (I/R) in mammalian models of I/R in the heart, liver, brain and kidney. Interestingly, analogous to the ischemic preconditioning cell survival program, nitrite administered locally during I/R or to the whole animal 24 hours before an ischemic episode mediates significant cytoprotection. The mechanism underlying both the acute and preconditioning cytoprotective effects of nitrite is unknown. Here we show that nitrite-dependent cytoprotection may be due to the prevention of I/R-induced mitochondrial dysfunction. Using an in vitro assay to model I/R injury in isolated mitochondria, we show that isolated mitochondria subjected to anoxia for thirty minutes sustained a fifty percent drop in complex II dependent respiratory rate at reperfusion, indicative of mitochondrial damage. However, mitochondria isolated from rats that received one bolus injection of nitrite (48 pmol – 480 nmol) 24 hours prior to mitochondrial isolation or nitrite (1- 100 µM) during the anoxic period were protected from the decrease in respiratory rate, suggesting that nitrite mediates both delayed and acute protection even at the mitochondrial level. Mechanistically, we show data demonstrating that nitrite modifies thiols on complex I by post-translational S-nitrosation to inhibit its activity. This dampening of electron transfer through complex I effectively decreases reactive oxygen species generation by the mitochondrion at reperfusion, thus protecting complex II through IV from oxidative damage and preventing cytochrome c release from the mitochondria. These data suggest that nitrite-dependent protection of tissue from I/R injury is at least partially due to nitrite-dependent modulation of mitochondrial function. Furthermore, these data highlight parallels between nitrite-dependent cytoprotection and ischemic preconditioning and suggest that nitrite may represent an effector molecule of the ischemic preconditioning cell survival program.

S31C
CARDIOVASCULAR EFFECTS OF DIETARY NITRATE
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Among the general public nitrate and nitrite are mostly known as unwanted residues in the food chain with potential harmful effects. For biologists and biochemists these inorganic anions have universally been viewed as inert metabolic end

products of endogenous NO oxidation. These views are now changing dramatically and we are on the edge of a veritable paradigm shift. From intense research over the past decade it is now clear that nitrate and nitrite can recycle in vivo via several biochemical pathways and again form bioactive NO. In fact the nitrate-nitrite-NO pathway is now emerging as an important complement to the classical L-arginine/NO synthase pathway for the generation of NO in blood vessels and tissues. This has important physiologic as well as therapeutic implications. The findings of potent cardiovascular effects of nitrate have intriguing dietary implications. It is known that a diet rich in vegetables lowers blood pressure and protects against cardiovascular disease, but the active ingredient(s) are still unknown. I will discuss the evidence that inorganic nitrate - via conversion to nitrite and vasodilatory NO – is beneficial for the cardiovascular system.

S31D

CHRONIC SODIUM NITRITE THERAPY AUGMENTS ISCHEMIC TISSUE ANGIOGENESIS AND BLOOD FLOW.

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It has recently been shown that nitrite functions as a hypoxia-activated Nitric Oxide (NO) donor that can protect tissues from acute ischemia-reperfusion injury in the heart and liver. However, the effects of nitrite therapy in the setting chronic vascular disease have not been studied. Our laboratory is probing the hypothesis that continuous sodium nitrite therapy during chronic ischemia can augment angiogenic activity in an NO-dependent manner. Chronic ischemia is induced in the mouse left hind limb via femoral artery ligation with the contralateral limb remaining intact. Sodium nitrite was administered intraperitoneally at a range of doses between 8.25 µg/kg to 3.3mg/kg twice daily over a 7-day period. Continuous sodium nitrite therapy significantly increased ischemic hind limb blood flow (mL/min/100g tissue) as measured by laser Doppler with lower doses of sodium nitrite being more effective than higher doses. Moreover, sodium nitrite restoration of ischemic tissue blood flow occurred rapidly within 3 days of ischemia. Sodium nitrite also significantly enhanced ischemia induced angiogenesis and endothelial cell proliferation in a similar fashion compared to PBS control treatments. Importantly, sodium nitrite had minimal effects on non-ischemic hind limb blood flow and did not alter angiogenic activity in the non-ischemic hind limb. Sodium nitrite therapy also increased ischemic tissue nitrite, SNO, and XNO levels compared to non-ischemic limbs but decreased plasma levels of nitrite possibly due to feed back inhibition of eNOS protein expression. Lastly, all of these responses were nitric oxide dependent as the NO scavenger Carboxy PTIO (1 mg/kg) abolished the beneficial effect of sodium nitrite in ischemic tissue. In conclusion, blood flow and angiogenic activity in chronically ischemic tissue is significantly enhanced by continuous low dose sodium nitrite therapy, which occurs through a nitric oxide dependent mechanism. These data strongly suggest that sodium nitrite therapy may serve as a novel therapeutic treatment for peripheral artery and critical limb ischemic diseases.

Friday, August 29th
Cell adhesion under flow
Parallel Symposium – S32

S32A

ACTIVATION-INDEPENDENT PLATELET ADHESION AND AGGREGATION UNDER ELEVATED SHEAR STRESS

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Platelet aggregation, which contributes to arrest bleeding but also to thrombovascular disorders, is thought to initiate after signaling-induced activation. We found that this paradigm does not apply under blood flow conditions comparable to those existing in stenotic coronary arteries. Platelets interacting with immobilized von Willebrand factor (VWF) aggregate independently of activation when soluble VWF is present and the shear rate exceeds 10,000 s^{-1} (shear stress 400 dyn/cm^2). Above this threshold, active A1 domains become exposed in soluble VWF multimers and can bind to glycoprotein Ibα promoting additional platelet recruitment. Aggregates thus formed are unstable until the shear rate approaches 20,000 s^{-1} (shear stress 800 dyn/cm^2). Above this threshold, adherent platelets at the interface of surface-immobilized and membrane-bound VWF are stretched into elongated structures and become the core of aggregates that can persist on the surface for minutes. When isolated dimeric A1 domain is present instead of native VWF multimers, activation-independent platelet aggregation occurs without requiring shear stress above a threshold level, but aggregates never become firmly attached to the surface and progressively disaggregate as shear rate exceeds 6,000 s^{-1}. Platelet and VWF modulation by hydrodynamic force is a mechanism for activation-independent aggregation that may support thrombotic arterial occlusion.

S32B

FORCE REGULATED LFA-1-ICAM-1 INTERACTIONS IN LYMPHOCYTE CROSSING OF VASCULAR ENDOTHELIUM

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Endothelial displayed chemokines play a key role in the arrest, crawling and transendothelial migration (TEM) of leukocytes. Specialized machineries activate the key lymphocyte integrin LFA-1 to coordinate these sequential processes. Lymphocyte crawling over apical endothelial surfaces and TEM are both LFA-1 dependent. Lymphocytes rapidly arrest on activated endothelial cells via subsecond activation of their VLA-4 and LFA-1 integrins by endothelial displayed chemokines and the Rap-1 GTPase GEF, CalDAG-GEFI is critical for this chemokine induced events underlying lymphocyte arrest. During crawling, chemokines rearrange high affinity LFA-1 with endothelial ICAM-1 in numerous scattered submicron dots, and form adhesive filopodia, some of which invade into the endothelial cells body even prior to TEM. In the presence of shear force and high level of chemokine signals, the number of adhesive and invasive filopodia is greatly enhanced. Lymphocyte crossing through the endothelial barrier also requires shear flow signals and involves the formation of large basolateral lamellipodia enriched with ICAM-1 occupied high affinity LFA-1. We propose that chemokine triggered force activated LFA-1-ICAM-1 bonds mediate lymphocyte diapedesis by coordinating short lived focal attachments and protrusions through the serial activation of high affinity LFA-1 dots underneath transmigrating lymphocytes.

S32C

NEUTROPHIL ADHESION UNDER SHEAR IN VIVO AND IN WHOLE BLOOD FLOW CHAMBERS

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Blood neutrophils are adapted to adhesion under flow. Isolated human neutrophils in parallel plate flow chambers can roll and adhere at low shear stress (1 dyn/cm2), and most adhesion is lost at 2 dyn/cm2. However, physiologic shear stresses in vivo range above 10 dyn/cm2, and neutrophils in whole blood can roll and adhere on E-selectin or P-selectin at up to 20 dyn/cm2. Rolling neutrophils sense surface-immobilized chemokines through the chemokine receptors, which leads to immediate integrin activation and arrest. Neutrophils rolling on E-selectin are also activated through PSGL-1, which triggers tyrosine phosphorylation of the Src kinase Fgr and the ITAM adaptor proteins DAP-12 and FcRgamma. This results in activation of spleen tyrosine kinase Syk, which is required for partial activation of the integrin LFA-1. Partially activated LFA-1 supports neutrophil rolling at very slow velocities (typically less than 5 micrometers/s) and lowers the threshold for arrest by chemokines. Triggering this signaling pathway requires shear stress. In acute peritonitis, the PSGL-1 - Fgr - ITAM - Syk pathway and the chemokine receptor pathway each contribute about 50% to neutrophil recruitment and eliminating both almost completely blocks neutrophil recruitment. In conclusion, shear-dependent, PSGL-1-mediated activation is critically important for neutrophil trafficking in inflammation. Supported by US National Institutes of Health and German Research Foundation

S38E

PLATELET-MEDIATED ENHANCEMENT OF MONOCYTE ADHESION

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Recruitment of inflammatory cells in the perivascular tissue is a prerequisite for many physiological processes. While the inflammatory influx of leukocytes to combat infection near the entrance site of infective agents is protective, it gives rise to the development of diseases such as atherosclerosis, if continuous or perpetuated. We know from the multistep model that cellular recruitment consists of overlapping sequences initiated by capturing and rolling of the cell, followed by adhesion which is of paramount importance and naturally indispensable for subsequent transendothelial migration. The spectrum of molecules and cells involved in these processes is vast. Platelets have evolved in several ways as amplifiers of leukocyte adhesion, since beyond their function as cellular controllers of coagulation their repertoire of adhesion molecules and soluble mediators renders them important interplayers between endothelial cells and blood borne immune cells.

Friday, August 29th
Smart: small artery remodeling: a complex interplay between forces, matrix and cells
Parallel Symposium – S33

S33A
THE IMPACT OF CYCLIC STRETCH ON THE ADAPTIVE REMODELLING OF ARTERIOLES
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The enlargement and thickening of arterioles running in parallel to an occluded artery is an important adaptive remodelling process, which partially compensates the consequences of peripheral artery disease by creating natural bypasses. Although it has been shown that the change of biomechanical forces to which the vessel wall is exerted orchestrates its remodelling, the underlying mechanistic background is still poorly understood. In this context we identified cyclic stretch as an important trigger of the expression of MCP-1 in smooth muscle cells as well as ephrinB2 in endothelial cells, which are both involved in the recruitment and activation of monocytes during the adaptive remodelling of arterioles. In addition, ephrinB2 was found to control the migration of smooth muscle cells and may thus stabilize the architecture of the vessel wall during remodelling. Furthermore, we showed that the expression of both, MCP-1 and ephrinB2 is similarly regulated by the stretch activated transcription factor AP-1. Correspondingly, blocking of AP-1 completely inhibited the adaptive remodelling of collateral arterioles in vivo. Taken together, these results suggest that stretch induced activation of vascular cells strongly affects their functional contribution to adaptive remodelling of arteriolar vessel wall.

S33B
siRNA-MEDIATED DOWNREGULATION OF L-TYPE CALCIUM CHANNELS IN RAT MESENTERIC ARTERIES RESULTS IN UNEXPECTED REMODELLING
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Pharmacological intervention has demonstrated that Ca^{2+} entry via L-type voltage-gated Ca^{2+} channels (Cav1.2) is important for vascular contraction and vascular structure. In this study, we used siRNA applied *in vivo* to assess the importance of Cav1.2 for vascular structure and function. Branches of the mesenteric artery of anesthetized Wistar rats were transfected with siRNAs targeting Cav1.2 or with control siRNAs. The effect of transfection was analyzed using quantitative PCR and immunohistochemistry. The functional effects of transfection were studied using isometric myography. Transfection downregulated mRNA of Cav1.2 by 93±2% within 3 days while the mRNA level was recovered 10 days after transfection (110±13 %). Protein expression was also reduced after 3 days. *In vivo* the diameter was increased as expected but *in vitro* under relaxed conditions the diameter was surprisingly reduced by 33±10% (n=7). The force development to 125 mM K^+ was reduced (by 72±10%, n=6). The responses to agonists were also suppressed in Cav1.2 downregulated arteries. The reduced force development was accompanied with reduced nifedipine sensitive $[Ca^{2+}]_i$ increase. Transfection of arteries *in vivo* with siRNA demonstrated the importance of Cav1.2 for vascular structure and reactivity and its importance for SMC Ca^{2+} handling.

S33C
VASCULAR INTEGRINS AS MODULATORS OF ENDOTHELIAL CELL FUNCTIONS
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Integrins are cell surface receptors mediating cell adhesion the extracellular matrix and initiating signaling events necessary for migration, proliferation and survival. Signaling pathways activated by integrins include MAPK, PI-3K, PKB/Akt, Rho family GTPases and NF-kappaB. Besides their role in vascular development and angiogenesis, integrins also play a critical role in regulating vascular homeostasis in response to microenvironmental cues. For example, integrin-ligation determines the endothelial response to tumor necrosis factor (TNF) by acting permissive for TNF-induced PKB/Akt. NF-kappaB activation by TNF does not require integrin ligation. Concomitant activation of PKB/Akt and NF-kappaB is essential for the survival of TNF-exposed endothelial cells. Attenuation integrin ligation prevents TNF-induced PKB activation, resulting in increased cell death. Thus, vascular integrins regulate inflammatory *vs* apoptotic response to TNF. Ligation of soluble high-affinity ligands to non-engaged alphaVbeta3 integrin, an integrin expressed on tumor vessels, results in integrin activation, recruitment to cell-cell contacts, and transient disappearance of VE-cadherin from intercellular junctions. In a tumor situation, treatment with a high-affinity integrin ligand facilitates the accumulation of cytotoxic drugs in the tumor, consistent with increased vascular permeability upon disruption of VE-cadherin-mediated cell-cell adhesion. Integrins are important modulators of endothelial cell responses during physiological and pathological situations and integrin modulation may open new therapeutic opportunities.

S33D

CELLS AND FORCES: ROLE OF THE ACTIN CYTOSKELETON IN REMODELING

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Vascular remodeling occurs in response to altered mechanical stress on the vessel wall. Due to the law of Laplace, vasorelaxation at constant transmural pressure causes increased wall stress, while contraction alleviates stress. Vascular tone and remodeling are thus intimately connected. While shear forces on the endothelium lead to relaxation, stretch of the vascular smooth muscle cells may raise myogenic tone and also cause increased protein synthesis and contractility. Actin polymerization is crucial for the expression of genes regulated by serum response factor (SRF) and the cofactors myocardin and MAL/MRTF. This group of genes is associated primarily with the contractile and cytoskeletal systems. Their expression marks the contractile phenotype of quiescent smooth muscle cells as opposed to the synthetic phenotype of proliferating cells, which is typically seen in cell culture. The contractile phenotype can however be maintained in organ culture of intact vascular preparations, which also allows stretch of the vascular wall. When stretch is applied on mouse portal vein in culture, focal adhesion kinase (FAK) is biphasically phosphorylated, correlating with rapid (minutes) MAP kinase activation and slower (hours) Rho activation, stimulating actin polymerization. The result is synthesis of smooth muscle marker proteins and growth of the vascular media with cells maintained in contractile phenotype.

S33E

TRANSGLUTAMINES IN SMALL ARTERY REMODELING

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Transglutaminases cross-link proteins such as fibronectin and collagens, and thereby stabilize extracellular matrices. We hypothesized that remodeling of small arteries depends on transglutaminases. Small mesenteric arteries from mice express type 1 and type 2 transglutaminase, and the plasma transglutaminase, Factor XIII. Exposure of arteries to exogenous transglutaminase induces inward remodeling. In addition, manipulation of transglutaminase activity and expression during organ culture of small arteries results in vascular remodeling. To study the in vivo relevance of transglutaminases, mice deficient for type 2 transglutaminase were subjected to surgically altered blood flow. In these mice the inward remodeling in response to low blood flow was delayed, but not absent. Factor XIII derived from attracted macrophages appeared to compensate for the loss of type 2 transglutaminase. As a second in vivo model of small artery remodeling, the type 2 transglutaminase knockout mice were subjected to L-NAME-induced hypertension. The increase in blood pressure was similar in knock-out as compared to wild-type littermates. However, inward remodeling was delayed in the knock-out mice and wall stress remained increased. In conclusion, these results suggest inward remodeling of resistance arteries depends on transglutaminases. This research is supported by the Netherlands Heart Foundation, grants 2001D038 and 2005B080.

Friday, August 29th
Non-invasive oxygenation measurements: applications, limitations and perspectives
Parallel Symposium – S34

S34A
MEASUREMENT OF OXYGEN IN THE MICROCIRCULATION USING PHOSPHORESCENCE QUENCHING MICROSCOPY (PQM)
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The transport of oxygen is one of the important functions of the microcirculation. Quenching of phosphorescence from an excited phosphor by oxygen provides a means to measure oxygen in microvessels or interstitial fluid. The rate of phosphorescence decay is related to PO_2 by the Stern-Volmer equation. To achieve adequate signal/noise, implementation of PQM requires higher light intensity and phosphor concentration than used in macro-scale applications. When the phosphor is excited, it can decay back to the ground state by emitting light (phosphorescence) or by transferring energy to oxygen (collisional quenching), creating the highly reactive species singlet oxygen, thereby consuming oxygen. It is important to minimize this consumption artifact by reducing excitation intensity and frequency and the size of the excitation region. Major findings using PQM are (1) the first measurements of Erythrocyte Associated Transients (EATs) in PO_2 as RBCs and plasma gaps alternately pass an observation point in a capillary, (2) observation of a small (1-2 mmHg) transmural PO_2 gradient in arterioles, (3) observation of a small PO_2 gradient in the interstitial fluid, and (4) measurement of VO_2 in the tissue using the stop-flow method. PQM has proven to be a robust method for measuring oxygen levels in the microcirculation. Supported by grants HL18292 and HL079087 from the NHLBI.

S34B
TISSUE OXYGEN DISTRIBUTIONS MEASURED BY PHOSPHORESCENCE QUENCHING: INTRAVASCULAR, INTERSTITIAL AND INTRACELLULAR SPACES
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Oxygen dependent quenching of phosphorescence is a minimally invasive, real time, optical method for measuring oxygen that can be used measure oxygen in many different applications. Phosphorescent oxygen sensors have been designed and synthesized that have absorption and emission in the near infrared region of the spectrum, are soluble in the body fluids and biologically inert. Oxygen pressures can be measured in any optical measurement mode, individual points, 2-D imaging, and 3-D imaging. The oxygen distribution in individual tissue compartments of both normal and pathological tissue in mice, rats and pigs. In normal tissue, there is little difference in lowest 10% of the oxygen pressures between the intravascular and interstitial spaces (capillary bed) in resting muscle or brain (<1.5 torr). The oxygen distribution in the vascular space has a component where the oxygen pressures are substantially higher than in the surrounding interstitial space (arterioles and veins). The absolute values for the oxygen pressures are dependent on the physiological condition (such as awake or anesthetized, the anesthetic used and its dose, etc.). Increased work rate (exercise in muscle) and tissue pathology (tumors) alter the oxygen distributions in a manner consistent with the changes in oxygen consumption and/or vascular density. Supported by NS-31465, HL081273, and U54 CA105008.

S34C
MICROVASCULAR HEMOGLOBIN OXYGEN SATURATION MEASUREMENTS USING RAMAN MICROSPECTROSCOPY
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Studies from our group have shown that Raman microspectroscopy can be used for noninvasive measurements of hemoglobin oxygen saturation (SO2) in the microcirculation (Am J Physiol 289:H488,2005; Analytical Chemistry 79:1514,2007; J Appl Physiol,2008). The resonant Raman enhancement of hemoglobin is valuable in structural and ligand binding assessment of heme proteins, allowing simultaneous identification of oxy and deoxy species of hemoglobin. Using laser excitation at 406 or 532 nm, back scattered Raman light from selected regions was collected by a microscope objective and directed to a spectrometer connected to a detector. The intensities of oxy and deoxy heme spectral peaks were used to determine the relative concentration of each species. Calibration *in vitro* was performed using blood flowing through glass capillaries at oxygen tensions. Fluorescence did not significantly degrade the signal quality and the estimated SO2 using Raman correlated well with SO2 measured via co-oximetry. Spectral deconvolution was also used to accommodate for the effects of overlapping bands on signal intensities. Since the system includes phosphorescence-quenching methodology,

measurements of oxygen partial pressure and SO2 can be made on the same location using intravital microscopy. The methodology can be further developed to provide noninvasive measurements of SO2 of the human mucosa. Support: ONR and NIH (HL079087).

S34D

MAPPING OF OXYGEN SATURATION DURING INTRAVITAL MICROSCOPY

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Uptake of oxygen in the lung and delivery is a key microvascular function. Spectroscopic methods have been used to estimate oxygen saturation (SO_2), and the introduction of multi-wavelength imaging systems for light microscopy allowed the application of this approach to intravital microscopy (IVM). The aim of our development was to provide a tool for high resolution 2D imaging of SO_2 in different tissues. Standard imaging hardware (TILL Photonics) was used to record wavelength stacks (500-600nm) of images from a given field of view during IVM. These stacks were analyzed using a multispectral approach (SOAP, Styp-Rekowska *et al*., Microcirculation 2007) to obtain oxygen saturation and hematocrit. References for oxygenated and desoxygenated hemoglobin were obtained from blood perfused microtubes and from in vivo recordings. Using a correction for tissue movements, SO_2-values were obtained with a spatial resolution of about 1 microm. Vessels down to capillary size can be analyzed, and intravascular oxygen saturation gradients are seen in venules. Since the system works well with trans- and epillumination it can be applied to a large variety of tissues including a specially adapted adaptation of murine lung model. The implementation of spectral analysis to IVM combining available multi-wavelength systems with custom analysis software generates a tool for high-resolution 2D imaging of SO_2 in the microcirculation.

Friday, August 29th
The role of platelets in hematopoietic stem cell trafficking
Parallel Symposium – S35

S35A
HOMING OF STEM CELLS TO A VASCULAR INJURY: A MATTER OF SEEDING IN FERTILE SOIL
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A vascular injury leads to the establishment of a very specific injury-associated micro-environment, consisting of activated platelets, platelet-secreted factors, factors secreted by the injured endothelium and end-products of the coagulation cascade. We have identified activated platelets as being crucial for the initial tethering of $CD34^+$ cells to the site of endothelial damage, fibrin as supporting factor that mediates firm adhesion and both fibrin and activated platelets as factors promoting commitment of the stem cells towards an endothelial-like phenotype (ATVB 2006 Jul;26(7):1653-9). Thus, these hemostatic components play a role in stem cell-mediated vascular repair.
We make use of an *ex vivo* flow-model, consisting of a monolayer of TNF-stimulated endothelial cells, covered with fibrin-rich platelet thrombi, thereby mimicking the injury-associated microenvironment. This flow-model enables us to study specific compounds of the microenvironment in detail. Furthermore, once $CD34^+$ cells are immobilized at the injury-site, they become subject to shear. We show that shear can rapidly upregulate VEGFR-2 expression on $CD34^+$ cells, while non-sheared cells do not exhibit VEGFR-2 expression. VEGFR-2 originates from an intracellular compartment, where also CXCR4 is localized. Therefore, shear liberates both VEGFR-2 and CXCR4, making the cells sensitive to VEGF and SDF within minutes. Supported by The Netherlands Heart Foundation (Grant 2005B106).

S35B
AMBIVALENCE OF PROGENITOR CELLS IN VASCULAR REPAIR AND PLAQUE STABILITY
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The mobilization and recruitment of bone marrow-derived or resident progenitor cells giving rise to smooth muscle-like cells have been implicated in neointima hyperplasia after arterial injury and in accelerated forms of arterial lesion formation, e.g. transplant arteriopathy or graft vasculopathy. By contrast, convincing evidence has emerged that the arterial homing of endothelial progenitor cells contributes to endothelial recovery and thereby limits neointima formation after endothelial denudation or stent implantation. In the chronic context of primary atherosclerosis, plaque progression and destabilization, a more complex picture has become apparent. In patients, the number and function of endothelial progenitor cells have been linked with an improved endothelial function or regeneration but have been inversely correlated with cardiovascular risk (factors). In animal models, however, the injection of bone marrow or endothelial progenitor cells or the application of stem-cell mobilizing factors have been associated with an exacerbation of atherosclerosis and unstable plaque phenotypes, whereas the contribution of bone marrow-derived smooth muscle progenitors to primary atherosclerosis appears to be rather confined. This paper intends to discuss crucial cues, namely chemokines, adhesion molecules, growth factors and pharmacological means that guide and control the context-specific mobilization, recruitment and fate of progenitor cells in arterial disease.

S35C
TRAFFICKING OF HEMATOPOITIC STEM AND PROGENITOR CELLS
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Blood cell homeostasis depends on a rare population of precursor cells, the hematopoietic stem and progenitor cells (HSPCs), which possess the capacity for self-renewal and multilineage differentiation. HSPCs are thought to reside mostly in specialized niches in bone marrow (BM) cavities. Nonetheless, a considerable number of HSPCs constitutively migrates out of the BM and enters the blood. The residence time of individual HSPCs within the blood is in the order of minutes, implying that the turnover of HSPCs that enter and leave the bloodstream must be high. However, the ultimate fate and functional relevance of these circulating HSPCs is largely unknown. We have shown that HSPCs constitutively survey extramedullary non-lymphoid tissues. Under physiological conditions migratory HSPCs contribute to the continuous restoration of specialized hematopoietic cells that reside in peripheral tissues. During infection and inflammation, the migratory pool of HSPCs acts as an immediate and highly adaptive source of progenitor cells that proliferate locally and generate innate immune effector cells. Platelets secrete several chemotactic mediators that control the migration of HSPCS. Hence, platelets, which are among the first cells to arrive at the scene of vessel damage, have the ability to direct HSPCS to sites of tissue injury.

S35D
CRITICAL ROLE OF PLATELETS IN THE POSTNATAL CLOSURE OF THE DUCTUS ARTERIOSUS BOTALLI

K Schürzinger [1], Konstantin Stark [1], Michael Lorenz [1], Sandra Kerstan [1], Lena Orschiedt [1], Martina Rudelius [2], Stefan Seidel [2], Luise Jennen [3], Axel Walch [3], Elisabeth Kremmer [4], Steffen Massberg [4]
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The Ductus arteriosus Botalli (DA) is an arterial shunt vessel directing the blood away from the non-ventilated fetal lung toward the aortic arch. While patency of the DA is vital for normal fetal development, the DA closes within few hours after birth. Persistent patent DA (PDA) is a major cause of morbidity in neonates. Postnatal occlusion of the DA is initiated by DA constriction. However, the subsequent processes leading to intimal reorganization and eventually definite DA closure are mostly undefined. The goal of our present study was to define the role of platelets in postnatal DA closure in a neonate mouse model.
First, we asked whether platelets are indeed recruited to the DA perinatally. In fact, immunohistochemistry using platelet-specific anti-CD41 antibodies and electron microscopy revealed accumulation of platelet within the DA lumen. Next, we addressed potential platelet-endothelium interactions in the DA using a novel model of intravital videofluorescence microscopy in a neonate mouse model. We observed that within the first hour after birth, platelet adhesion and aggregation is a prominent phenomenon within the DA of the anaesthetized, mechanically ventilated neonate mouse. Finally, we analysed the functional role of platelets in DA closure in mice. Twelve hrs after birth, wild type (WT) pups, pups treated with function-blocking anti-GPVI mAb, GPIIb-IIIa-deficient pups or NF-E2-deficient pups with severe platelet deficiency, were subjected to angiography to evaluate closure or patency of the DA. In all WT pups, occlusion was complete 12 hrs after birth. In contrast, inhibition of GPVI prevented term DA-closure in 54% of the pups (n=7/13). Likewise, loss of GPIIb or platelet deficiency (NF-E2-/- mice) resulted in DA patency in 31% (n=5/16) or 69% (n= 4/13) of the pups, respectively. Interestingly, we observed a significant reduction of nucleated, intimal cells within the DA in neonates with loss/dysfunction of platelets compared to WT pups. Additionally, the blockade of SDF-1a, known to mediate platelet-assisted cell recruitment to vascular lesions, reduced term closure of the DA in 50% (5/10) of 12hrs old neonates.
The findings show for the first time that platelet adhesion plays an essential functional role in appropriate DA closure and intimal DA reorganization. The data demonstrate in vivo that platelets are recruited in the DA, induce thrombotic occlusion of the vessel and modulate the accumulation of intimal cells during postnatal DA remodelling.

Friday, August 29th
Metabolism and endothelium
Parallel Symposium – S36

S36A
OXYGEN SENSING IN ENDOTHELIUM
G Breier, AK von Ameln, Z Kirsnerova, I Prade, A Licht, A Gembarska, K Anastassiadis, F Stewart, J Kalucka, B Wielockx
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All higher organisms possess mechanisms to maintain oxygen homeostasis. Low oxygen levels, or hypoxia, induce a variety of compensatory reactions, including enhanced erythropoiesis and angiogenesis. The cellular response to hypoxia is mediated by transcriptional regulators called hypoxia-inducible factors (HIFs). HIF stability, in turn, is regulated by HIF prolyl hydroxylases (PHDs), enzymes that function in cellular oxygen sensing. We are studying the function of HIF and of HIF-hydroxylases during physiological and pathological angiogenesis, with specific focus on their function in endothelial cells. Using a dominant-negative approach we could show that HIF activity in endothelial cells is essential for mouse cardiovascular development; HIF inhibition led to impaired vascular remodelling, angiogenic sprouting, and heart looping. Yet, the retrovirus-mediated inhibition of HIF activity in tumor vessels accelerated tumor growth. Overexpression of PHD2 or PHD4 in tumor cells led to reduced HIF levels and slowed down the growth of experimental LM-8 osteosarcoma in mice. Analysis of tumor sections revealed alterations in vessel structure and function. PHD-2 deficient mouse embryos die in utero, showing that PHD2 has essential functions during embryonic development. Our data implicate endothelial HIF activity in physiological and pathological angiogenesis and indicate that PHDs play an important role in tumor progression. Supported by grants from the Bundesministerium für Bildung und Forschung (BMBF) and the Deutsche Forschungsgemeinschaft (DFG)

A36B
NEW CONCEPTS ON THE PROTECTIVE EFFECTS OF SHEAR STRESS
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Shear stress, the frictional force due to blood flow, is an important determinant of vascular homeostasis, exerting potent anti-apoptotic and anti-atherosclerotic effects on endothelial cells (ECs). This is evidenced by the strong correlation between atherosclerotic plaque localization and regional flow patterns: lesions form preferentially at arterial branch points and curvatures, where blood flow is turbulent or oscillating and shear stress is low. In contrast, vessel segments exposed to laminar blood flow and consequently high shear stress (10-15 dynes/cm^2) tend to remain plaque free. Although ECs respond to changes in local shear rates by modulating the induction and/or repression of several genes, the beneficial effect of shear stress is largely due to local release of nitric oxide (NO), produced by the endothelial NO synthase (eNOS). Opposite to the NO/NO synthase system, the renin-angiotensin system, that participates in vasoconstriction and arterial remodelling, has rather pro-atherosclerotic effects in the vascular wall. We recently found that shear stress may counter these effects by downregulating angiotensin type 1 receptors (AT1R). En face immunohistochemistry revealed positive endothelial AT1R staining only in the inner regions of the aortic arch and at collateral artery branch points of C57BL/6 mouse aortas, where blood flow is low. We also observed a biphasic decrease in AT1R protein expression in ECs exposed to a laminar shear stress, which was dependent on NO and protein kinase G, leading to transcriptional downregulation of AT1R. Hence, shear stress may convey some of its atheroprotective effects through degradation and downregulation of AT1R in endothelial cells.

S36C
ROLE OF T-CELLS IN THE GENERATION OF OXIDATIVE STRESS IN ENDOTHELIUM
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Oxidative stress promotes atherosclerosis contributes to cardiovascular morbidity and mortality. Similarly inflammation plays a key role in the pathogenesis of most of cardiovascular disorders. The link between the two is however poorly understood. We have recently shown that mice lacking T and B cells are protected against vascular oxidative stress induced by systemic angiotensin II infusion or desoxycorticosterone acetate (DOCA) – salt. Importantly this effect on vascular oxidative stress is accompanied by loss of hypertension and vascular dysfunction in these models. Adoptive transfer of T, but not B, cells restored these abnormalities. Angiotensin II is known to stimulate reactive oxygen species production via the nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase in several cells, including some immune cells. Accordingly, adoptive transfer of T cells lacking the angiotensin type I receptor or a functional NADPH oxidase resulted in blunted angiotensin II – dependent hypertension and decreased aortic superoxide production. Angiotensin II increased T cell markers of activation and tissue homing in wild-type, but not NADPH oxidase – deficient, mice. Angiotensin II markedly increased T cells in the perivascular adipose tissue (periadventitial fat) and, to a lesser extent the adventitia. These cells expressed high levels of CC chemokine receptor 5 and were commonly double negative (CD3+CD4-CD8-). This infiltration was associated with an increase in intercellular adhesion molecule-1 and RANTES in the aorta. Hypertension also increased T lymphocyte production of tumor necrosis factor alpha, and treatment with the TNF alpha antagonist etanercept prevented the hypertension and increase in vascular superoxide caused by angiotensin II. These studies identify a previously

undefined role for T cells in the genesis of vascular oxidative stress in hypertension and support a role of inflammation in the basis of these vascular abnormalities. T cells might represent a novel causative therapeutic target for the treatment of vascular oxidative stress and hypertension.

S36D

RELEASE OF ATP VIA CONNEXIN 43 PREVENTS ENDOTHELIAL BARRIER FAILURE INDUCED BY HYPOXIA-REOXYGENATION

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Reperfusion of the ischemic heart causes endothelial barrier failure leading to edema and jeopardizes recovery of the reperfused organ. Recently, we have shown that extracellular ATP, spontaneously released from coronary endothelial cells during reperfusion, protects the barrier against reperfusion injury. Here we analyzed, whether connexin 43 (Cx43) mediates ATP release during hypoxia and reoxygenation (H/R) in endothelial cells (EC) and whether this Cx43-mediated release prevents endothelial barrier failure induced during H/R. To downregulate Cx43 EC were transfected with Cx43 non-silencing (control) or silencing siRNA 24 hrs before onset of H/R, which leads to an 60-70% reduction of cellular Cx43 content (western blot analysis). EC respond with a transient ATP release at the onset hypoxia as well as reoxygenation as determined in the EC supernatants using a luciferin-luciferase assay. Western blot analysis of the corresponding cells showed a close correlation between ATP release and cellular Cx43 content. Cx43 silencing or pre-incubation of EC with the Cx inhibitor 18-α glycyrrhetinic acid abolished the H/R-induced ATP release. Furthermore, H/R-induced endothelial barrier failure (formation of intercellular gap) was enhanced in Cx43 downregulated EC. Conclusion: During H/R ATP is released from endothelial cells by a Cx43-dependent mechanism and protects endothelial barrier function.

Friday, August 29th
Physiological shear stress- dynamic approach for monitoring drug efficacy in cell adhesion
Parallel Symposium – S37

S37A

DIFFERENTIAL CELL ADHESION WITHIN AN ISOGENIC MODEL OF MELANOMA PROGRESSION UNDER SHEAR FLOW CONDITIONS USING A MICROFLUIDIC CELL-BASED ASSAY

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Steps in the metastatic process include intravasation, survival in circulation, arrest in a specific organ, extravasation, growth and secondary tumour formation. In this study, we modelled survival in circulation, adhesion to endothelial cell-derived proteins, as well as to endothelial cells, using Cellix's VenaFlux™ Platform, to determine if the steps in extravasation differed in an isogenic melanoma cell line model of progression. The isogenic cell line model series was comprised of the poorly tumourigenic parental cell line, WM793, and its derivatives, WM793-P1, WM793-P2 and 1205-Lu (the latter obtained from a spontaneous metastasis to the lung). We found that WM793, WM793-P1 and WM793-P2 cells did not adhere to VCAM, ICAM or fibronectin, whereas 1205-Lu cells adhered to VCAM under defined flow conditions. To examine this further, we applied a decreasing gradient shear stress of 5, 2, 1, 0.5, 0.25 and 0.1 dyne/cm^2, which resulted in increasing adhesion of 1205-Lu cells to VCAM at shear stresses lower than 2 dyne/cm^2. GFP-labelled WM793 and 1205-Lu cells were flowed over endothelial cells at defined shear stress; again, adhesion could only be observed in case of 1205-Lu. The VenaFlux™ platform is a useful and reliable *in vitro* screening tool for key cellular phenotypes relevant to metastasis.

S37B

PHARMACOLOGICAL INVESTIGATION OF LEUKOCYTE ADHESION USING A NOVEL MICROFLUIDICS PLATFORM

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The inhibition of leukocyte recruitment to vascular endothelium represents an attractive target for the potential treatment of atherosclerotic cardiovascular disease. In particular, drugs that target either GPCRs or integrins on the surface of leukocytes are being investigated in pre-clinical discovery programmes. In order to evaluate such compounds for effects on cell adhesion, there is a need for a robust secondary assay platform that allows measurement of compound potency in either recombinant or native cells under flow conditions. One such platform has been developed by Cellix, utilising microfluidics to analyse leukocyte recruitment to recombinant adhesion molecules immobilised on channels of a biochip. Examples of the use of this platform in drug screening cascades with isolated cells will be shown. In addition, the Cellix platform can be used to evaluate adhesion profiles in whole blood samples.
In this way, the platform could represent an important tool for the *ex vivo* evaluation of compound effects in pre-clinical animal models and for the evaluation of compound effects in dose-setting clinical trials. Preliminary evaluation of this type of application will also be presented.

S37C

CXCL8 INDUCED LOSS OF POLARITY AS A NEW STEP IN THE LYMPHOCYTE EXTRAVASATION PROCESS

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The mechanistic aspects of the multi-step process culminating in T-lymphocyte extravasation through the vascular endothelium are well established. However, the significance of the release of numerous chemokines from the endothelial surface during inflammation and the intravascular role of these proteins when presented in a non-immobilised form is poorly understood. We reintroduced chemokine treated T-cells to whole blood and registered the rheological vectors of the cells as they flowed through channels mimicking areas of localized vasodilation. We found that T-cells treated with CXCL8 became more spherical and were twice as likely to marginate to the edge of the channel when compared to non-treated cells. Furthermore, we present data indicating that cells which do not have a sufficiently spherical have difficulty rolling on selectins. Catch bond phenomena are also observed in T-cells at the critical shear stress at which extravasation is known takes place. At shear stresses where catch bonds were elicited there was an increase in the number of secondary capture events. Our data suggests a previously unidentified role for intravascular CXCL8 in the upregulation of the T-cell. This new role for a soluble chemokine could be interpreted as "step 0" in the already established multi-step adhesion cascade.

S37D

ASTHMA – PHARMACOLOGICAL INHIBITION OF HUMAN EOSINOPHIL ADHESION UNDER FLOW

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Inappropriate eosinophil infiltration of the lung followed by the release of their potent cytotoxic substances is key to the airway inflammation characteristic of asthma pathology. Numerous studies have demonstrated essential roles for cell adhesion molecules in selective eosinophil adhesion and transendothelial migration from the asthmatic lung microvasculature to the tissues. Using Cellix's microfluidic system a number of established drugs have been shown to inhibit human eosinophil adhesion under flow conditions. Clinically relevant concentrations of antihistamines (cetirizine, levocetirizine) or a leukotriene antagonist (montelukast) inhibited both unstimulated and GM-CSF-stimulated eosinophil adhesion to rhVCAM-1. The statins, fluvastatin and lovastatin, significantly inhibited GM-CSF-stimulated eosinophil adhesion to rhICAM-1 but had no significant inhibitory effect on unstimulated eosinophil adhesion. In contrast, mevastatin, simvastatin, and pravastatin had no significant effect on GM-CSF-stimulated eosinophil adhesion to rhICAM-1. None of the five statins tested significantly inhibited unstimulated or GM-CSF stimulated neutrophil adhesion to rhICAM-1 under flow conditions. Inhibition of eosinophil adhesion to VCAM-1 or ICAM-1 in conditions that mimic inflamed lung post-capillary venules at physiologically relevant concentrations indicate potential anti-inflammatory effects for these drugs independent of their known mode of action. These findings may inform the development of novel asthma therapy based on anti-eosinophil strategies.

Friday, August 29th
Flowmotion waves in laser doppler signals: the low frequency components.
Parallel Symposium – S38

S38A

PRESENCE AND SIGNIFICANCE OF SEVERAL LOW FREQUENCY COMPONENTS IN LASER DOPPLER BLOOD FLOW SIGNALS

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Laser Doppler flowmetry can be used for non-invasive monitoring of blood perfusion in arterioles and the capillary bed. The resultant signal is highly complex and contains several oscillatory components. In addition to the cardiac and respiratory oscillations (also propagating through the small vessels), several lower frequency components have also been identified, ranging from 0.007 to 0.1 Hz. All components vary slightly with time in both frequency and amplitude, thus presenting a very challenging task in terms of signal analysis. We will discuss the importance of time-frequency methods and logarithmic resolution, as well as length of recording and sampling frequency. The aim is for optimal decomposition of the signal into components that can be related to the specific physiological mechanisms involved in blood flow regulation within the microvasculature. Several studies investigating the physiological origins will be reviewed and current understanding will be outlined. Changes in the oscillatory components with ageing will be illustrated. The results of clinical studies of patients with cardiac failure, diabetes, and post-acute myocardial infarction, will be used to emphasize the potential of the approach.

S38B

MICROVASCULAR CORRELATES OF FLOWMOTION WAVES: FROM ARTERIAL DIAMETER CHANGES TO LASER DOPPLER FLOWMOTION. THE LOW FREQUENCY COMPONENTS IN LASER DOPPLER SIGNALS OF HYPERTENSIVE PATIENTS

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In experimental microvascular models, such as the hamster skin fold window preparation, the arteriolar rhythmic diameter changes were characterized and several frequency components (FCs) were computed, according to vessel branching orders. These FCs in the range 0.01 – 0.25 Hz were related to several factors, such as myogenic activity, respiratory and heart rates. Many FCs were evidenced in laser Doppler tracings derived from healthy volunteers and patients, corresponding to those detected in experimental animals. We studied the effects of short term therapy with the beta-blocker Nebivolol (5 mg/die for one month) on skin microcirculation in newly-diagnosed hypertension patients (HP). Twenty-eight subjects (20 males, 50.9 9.1 years old) were studied before and after treatment, compared with twenty-four healthy volunteers. Skin blood flow was significantly reduced in HP (6.9 2.1 PU vs 10.7 4.8 PU, $p< 0.01$) as well as the total power (1.18 1.02 PU^2 vs 3.5 2.6 PU^2, $p< 0.05$). The relative power spectral density (PSD) of low FCs (0.009 - 0.06 Hz) was reduced compared with the heart rate-related component. The endothelium-dependent and independent dilation, reduced in HP, recovered at the end of treatment as well as the total and relative PSD of low FCs.

S38C

LOW FREQUENCY FLOWMOTION WAVES IN POAD PATIENTS

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Endothelial, myogenic and sympathetic activities on microvessels sustain the skin microcirculation autoregulation. The break down of these mechanisms leads to gangrene in patients with peripheral arterial disease (POAD). With power spectrum analysis it is possible to assess the endothelial (0,009-002 Hz) the sympathetic (0.02-0.06 Hz), the myogenic (0.06-0.2 Hz) frequency components, the so called" slow flowmotion waves" (SFW) within the entire spectrum of flowmotion waves (0.009-1.6.Hz)

In a group of 20 Intermittent claudication (IC) and 20 Critical limb ischaemia (CLI) patients matched with a group of 20 healthy subjects (hs), we assessed the medium power spectrum of all flowmotion waves (0.009-1.6 Hz) and the prevalence of SFW (0.009-0.2 Hz)

Our results showed a significant reduction of all medium power spectrum (mps) of flowmotion waves in all the POAD patients vs hs, $p < 0.001$ (0,097 ± 0,020. 0,40 ± 0,10 vs 1.0 ± 0,5, respectively). The mps of SFW was particularly reduced in CLI.

Moreover, the SFW prevalence was markedly reduced by 70% ± 5% in CLI, while in IC the reduction was only by 20%± 4%. The sympathetic activity mps was not significantly reduced in CLI vs IC (0,45 ± 0,20 vs.0,54± 0,60).

In conclusion, our findings show that in POAD patients there is a decreased amplitude of all the flowmotion frequencies (0.009-1.6.Hz) in both IC and CLI patients while the reduction of SFW (0,009-002 Hz) seems to be marked only in CLI. These results are in accordance with clinical findings in POAD patients where the claudicant ones have less risk of gangrene because the autoregulative microvascular mechanisms are still present.

S38D

LINEAR AND NONLINEAR ANALYSES OF LASER DOPPLER FLOWMETRY SIGNALS RECORDED DURING ISOFLURANE-INDUCED ANAESTHESIA IN HEALTHY RATS

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Laser Doppler flowmetry (LDF) signals recorded in healthy rats are studied during anaesthesia induced by isoflurane, an anaesthetic commonly used in clinical practice. For this purpose, isoflurane is administered in thirty two rats. The doses of anaesthetic are chosen to obtain two groups of rats: a first one (sixteen rats) with light anaesthesia, and a second one (sixteen rats) with deep anaesthesia. The LDF probe is positioned on the rat thigh. The mean value of each recording is determined. The complexity of the LDF signals is also analyzed by computing their multifractal spectra. For the latter analysis, the processing method is first calibrated with an *a priori* known synthetic multifractal process. The results show that the average LDF mean value is lower in the group of deep anaesthesia than in the group of light anaesthesia. However, $P > 0.05$ when these differences are analyzed with the Mann-Whitney test. Moreover, depth of anaesthesia does not modify the weakly multifractal properties of the signals: average multifractal spectra of LDF signals in each group have the same width. These findings infer that isoflurane may change the microvascular tissues properties but these modifications have no influence on LDF signals complexity measured by multifractal spectra. The authors would like to thank the Perimed company for the loan of the blood flow monitor and probes.

POSTER PRESENTATION ABSTRACTS
Tuesday, August 26th
Angiogenesis
Poster Session - PAG

PAG1

HOW ARTERIES DIRECTLY CONTROL THE VENOUS PATTERN THROUGH MECHANICAL FACTORS DURING VASCULAR MORPHOGENESIS.

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The vascular network spanning the vertebrates' body is so finely branched that the positions of each vessel cannot be genetically encoded. However, in many cases, mature arteries and veins follow an exactly parallel path, which is often interpreted in favor of a genetic cross-talk between them during embryonic development. We present a detailed study of the spatio-temporal development of yolk-sac and brain arteries and veins in growing chicken embryos: a metamorphosis of the vasculature, from an organization in series to an organization in parallel, spontaneously occurs when the size of the tissue increases. Based on optical and local mechanical measurements, we demonstrate that this metamorphosis is due to the visco-elastic response of the tissue, which induces a local rearrangement of capillaries, leading to an increase of the hydraulic conductivity along paths strictly parallel to the growing arteries. These paths are further selected by blood flow, and subsequently remodeled into veins, when the size of the tissue becomes greater than a threshold, which has been confirmed using numerical simulations (electrical analogy) of idealized vascular configurations. Therefore, the developing arteries directly induce their mated veins by a mechanical, self-organized universal process of hemodynamic and visco-elastic origins.

PAG2

CHANGES IN IMMUNOPHENOTYPE, BEHAVIOUR AND MICROCIRCULATION OF HUMAN TUMORS IMPLANTED ON THE CHICK CHORIOALLANTOIC MEMBRANE

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The behaviour of a tumor and its metastasis are different concerning terapy response and prognosis because of an incomplete molecular characterization of the tumors and their microcirculation. Metastasis and their vascularization are not well understood. The chick embryo chorioallantoic membrane represents an experimental model for dinamic study of tumors metastasis and its angiogenesis. Our purpose was to study phentoypic changes of the human tumors and their vascularization at several passages on the chick CAM. Two bone metastasis (breast cancer and lung cancer), one ovarian tumor and one gastric carcinoma were studied. Imunophenotyping included cytokeratins, proliferation markers, vascular markers, VEGF and its receptors, AC 133 and TIE2. Bone metastasis survived for three passages on the chick CAM. Cytokeratin expression was lost at the first implant compared with initial tumor and few or no proliferation was observed in the tumor implant. A high proliferation of host blood vessels and a particular type of vascular anastomoses between host and graft blood vessels were observed. AC133, TIE2 + cells were detected in the wall of the tumor blood vessels. Our findings support a „personalized" immunophenotype for each tumor type and metastasis as a base for targeted therapy.

PAG3

ROLE OF EXTRACELLULAR RNA IN ARTERIOGENESIS

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Arteriogenesis describes the growth of functional collateral arteries from preexisting arterio-arteriolar anastomoses. Initial triggers are physical forces such as altered shear forces, which activate the endothelium. Inflammatory features of the endothelium are apparent by increased expression of adhesion molecules or the chemoattractant molecule MCP-1 leading to increased adhesion and migration of monocytes to the endothelium. Pretreatment of mice with RNase but not with DNase for 10 min before occlusion of the arteria femoralis significantly reduced the extent of arteriogenesis suggesting that extracellular RNA might play a role in the process of arteriogenesis. Accordingly, *in vitro* studies confirmed that RNA acts as a chemoattractant for monocytes. The migration of monocytes through an endothelial cell monolayer was increased by RNA to nearly the same extent as by MCP-1. Additionally, the adhesion of monocytes to microvascular endothelial cells was increased by extracellular RNA, but not by DNA. Furthermore, RNA increased the expression of ICAM-1 on endothelial cells, which was abolished in the presence of RNase. Extracellular RNA also leads to exocytosis of Weibel-Palade bodies, which

are storage granules for proinflammatory factors like P-selectin. Conclusively, proinflammatory effects of extracellular RNA might play a dominant role in arteriogenesis.

PAG4

IDENTIFICATION AND QUANTIFICATION OF SOLUBLE MEDIATORS DURING TUMOR PROGRESSION

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Signaling mediators can be secreted by virtually all types of cells and, depending on the particular microenvironment of a pathological scenario, in different amounts and combinations. Owing it to a lack of *in-vivo* investigative tools, our understanding on the manner the complex network of mediators act synergistically to coordinate cellular interactions in bringing about or alternatively, preventing, disease progression elude us.

In this study, we aimed to obtain signaling cues from the tumor microenvironment by isolating relevant interstitial fluid samples from this tissue. We use this approach to directly identify and quantify simultaneously a large panel of tumor soluble mediators, to estimate their dynamics at distinct stages during tumor growth and/or to investigate the efficacy of designated chemokines as therapeutic agents.

Experiments were performed in DMBA induced mammary tumors in rats. Animals were divided randomly in groups and continuous intravenous administration of GM-CSF, GM-CSF antibody or GRO-KC antibody was achieved via implanted osmotic pumps for 7 days.

Infusion of GM-CSF resulted in a reduced tumor growth-rate by about ~50% compared to untreated animals. Its administration however also increased significantly local tumor metastases. Strikingly, continuous infusion of an antibody to GM-CSF resulted in a similar reduction in tumor growth-rate but metastases were absent. Our results suggest that, after GM-CSF therapy, solid tumors and their stroma co-evolve and support each other during progression toward malignancy. The methodology applied here also allowed for the observation that GM-CSF administration parallels a marked reduction in GRO/KC within tumors. Continuous administration of an antibody against GRO/KC ligand without GM-CSF administration resulted in reduced tumor size of ~ 80% from baseline.

Taken together the methodology of this study offers an *in vivo* approach to system biology and provides a tool for identifying and arresting key mediators involved in tumor progression.

PAG5

ANGIOGENESIS, VEGF, ANGIOPOIETIN-2 AND TIE-2 EXPRESSION IN HUMAN BREAST LESIONS

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Angiopoietin-2 (Ang-2) mediates endothelial cell instability and promotes vascular leakage through binding to its receptor, Tie-2. In the absence of VEGF, Ang-2 promotes vessel regression, whereas in the presence of VEGF, Ang-2 promotes angiogenesis. However, the precise role of Ang-2 and Tie-2 in breast cancer progression, metastasis and angiogenesis remains unclear. Therefore we quantified and correlated CD31, VEGF, Tie-2 and Ang-2 expression in sections of normal human breast (n=6), benign (n=25) and pre-malignant hyperplastic tissue (n=19), and pre-invasive cancer (n=64).

Histological analysis revealed a significant increase in MVD between normal breast tissue and breast lesions (P=0.005) which was associated with a significant increase in cytoplasmic VEGF (P=0.005), Ang-2 (P=0.006), and Tie-2 (P=0.046) in breast epithelial cells. Moreover, Ang-2 expression in endothelial cells significantly increased with increasing severity of breast lesion (P=0.005), although endothelial Tie-2 and VEGF expression did not change.

These data suggest that in normal breast tissue where Ang-2 and VEGF levels are relatively low, Tie-2 acts to maintain vessel quiescence. The increased Ang-2 and VEGF production in pre-malignant and pre-invasive breast cancer cells suggests that these proteins bind to endothelial Tie-2 resulting in the increase in angiogenesis detected in the earliest stages of dysplasia. Supported by Yorkshire Cancer Research.

PAG6

EVALUATION OF NOVEL ANTIANGIOGENIC COMPOUNDS DERIVED FROM *IN SILICO SCREENING* USING AN ESTABLISHED VEGF RECEPTOR TYROSINE KINASE INHIBITOR AS LEAD STRUCTURE

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We used a conformational drug database („Super-Target“) to search for novel antiangiogenic compounds as innovative drugs for cancer treatment. Checking two- and three-dimensional structural similarities to the clinically relevant VEGFR tyrosine kinase (TK) inhibitor, vatalanib, as lead structure we identified 12 promising candidate drugs (HP-1 to -12). In vitro evaluation revealed 3 compounds (HP-2, -6, -7) with superior antiangiogenic, antiproliferative and antimigratory effects on primary liver cancer and endothelial cells, as compared to the lead structure. The most powerful compound, HP-2, is a highly specific VEGFR-2 TK inhibitor with an IC_{50} of < 5nM, which is 5-10-fold lower than those of vatalanib. Surprisingly, HP-2 was ineffective in inhibiting the closely related VEGFR-1 TK (>20µM). Modulation of signaling pathways (MAPK, AKT/mTOR, Jak/STAT) by HP-2 is currently under investigation. Preliminary data show that HP-2´s mode of action involves the induction

of cell cycle arrest and apoptosis, while unspecific cytotoxic do not contribute to the observed antineoplastic effects. Moreover, first animal studies confirm the good tolerability of HP-2 (50mg/kg) when given in 48h intervals. Our study shows that the identification of novel cancer drugs by use of *in silico* methods is a target-oriented and effective approach. The identified compounds are very promising and merit further elucidation.

PAG7

PROTEOMIC ANALYSIS OF BIOMECHANICALLY STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AS AN IN VITRO MODEL FOR THE INITIATION OF ARTERIOGENESIS

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Arteriogenesis, the positive outward remodelling and growth of pre-existent collateral vessels, has been shown to be initiated by alterations in blood flow whereby endothelial cells are exposed to increased fluid shear stress conditions. The underlying cellular mechanisms, inducing vascular proliferation and remodelling during the onset of arteriogenesis *in vivo* are far from being understood.

To understand the molecular events during biomechanical stimulation of endothelial cells, an *in vitro* approach was chosen, thereby exposing monolayers of human primary endothelial cells (human umbilical vein endothelial cells**,** HUVEC) to low (1dyne/cm^2) and high (30dynes/cm^2) laminar shear stress conditions. Proteomic analysis was performed to examine changes in protein expression of three biological replicas.

Based on two-dimensional gel electrophoresis, 61 candidate proteins were found with diverse spot intensity comparing both flow conditions. NanoLC ESI mass spectrometry analysis identified proteins functionally linked to organisation of the cytoskeleton, signal transduction pathways, protein degradation and metabolism.

In conclusion, our data show that different shear stress conditions acting on endothelial cells result in a number of deregulated proteins involved in cytoskeletal rearrangement, which are also found during arteriogenesis *in vivo*. Thus, the experimental setup may be used as a valid *in vitro* model of the early events of arteriogenesis.

PAG8

ICAM-1 CYTOPLASMIC TAIL REGULATES ENDOTHELIAL CELL REDOX STATUS THROUGH AN NADPH OXIDASE PATHWAY

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To investigate the hypothesis that ICAM-1 plays a role in VEGF induced angiogenesis through a redox regulation mechanism, a peptide containing the antennapedia cell permeable sequence (AP) or an AP peptide linked to the transmembrane and cytosolic tail of ICAM-1 were synthesized. Using various *in vitro* techniques, these two peptides were employed to measure alterations of redox status in cultured endothelial cells and to determine their biological effect. Treatment with AP-ICAM-1 significantly increased glutathione concentrations and glutamate-cysteine ligase (GCL) activity at 3, 6, and 8 hours with a peak level observed at 6 hours. Monitoring reactive oxygen species (ROS) production with DCF revealed a rapid increase in ROS generation over three hours following AP-ICAM-1 treatment. Treatment with apocynin, DPI, or Catalase attenuated AP-ICAM-1 dependent ROS production, whereas SOD treatment increased ROS production, implicating activation of NADPH oxidase. AP-ICAM-1 peptide was also found to impede the VEGF-A dependent chemotaxis of endothelial cells. Interestingly, buthionine sulfoximine treatment restored VEGF-A dependent cell motility illustrating the importance of AP-ICAM-1 augmentation of glutathione on endothelial cell chemotaxis. These data demonstrate that the cytoplasmic tail of ICAM-1 regulates the production of GSH through a redox sensitive pathway involving NADPH oxidase, which governs VEGF-A dependent chemotaxis. Funding Provided through NIH grant 5R01HL080482-02

PAG9

LYMPHANGIOGENESIS DETECTED BY D2-40, VEGF-C, AND VEGFR3 EXPRESSION IN MOLECULAR SUBTYPES OF BREAST CARCINOMA

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Few data are available about the lymphangiogenic profile of breast carcinoma, and virtually there are no data regarding the expression of lymphatic markers in the recently defined molecular types. Our purpose was to investigate the relation between lymphatic microvessel density (LMVD), expression of VEGF-C and VEGFR3, and molecular types of breast carcinoma defined by immunohistochemistry. There were investigated 96 patients with invasive breast carcinoma, stratified as basal-like, luminal (A and B), HER2 and unclassified, based on the expression of ER, PR, HER2, cytokeratin 5/6, EGFR, Bcl-2 and p53. Additional sections were stained for D2-40, VEGF-C and VEGFR. LMVD was calculated for peritumoral and intratumoral D2-40-positive. VEGF-C and VEGFR were scored based on the density and intensity found in tumor cells. Results were correlated with tumor stage, lymph node status and degree of differentiation (G). We found basal-like carcinoma in 12 cases, HER-2 type in 18, luminal A in 51, luminal B in 5, and unclassified in 10. The average of LMVD in the

peritumoral/intratumoral areas of each type was 7.62/2.58, 10.2/2.91, 10.7/4.42, 7.66/3.66, and 6.99/1/55, respectively. LMVD correlated with lymph node status (LNS) in luminal and HER2, but not in basal-like carcinoma. Lymphovascular invasion (LVI) was found in 23 (23.96%) cases on routine examination, and in 52 (54.1%) on slides stained with D2-40. VEGF-C expression in tumor cells did not correlate with LMVD and LVI, excepting for the HER2 subtype. VEGFR-3 expression in lymphatic endothelial and tumor cells was found in 59/96 cases and strongly correlates with LVI and LNS in all molecular subtypes excepting for the unclassified. No correlation was found between LMVD, VEGF-C and VEGFR3 expression and tumor stage and G. Our results suggest that VEGFR-3, but not LMVD and VEGF-C, accurately predicts LNS and could be an attractive target for specific therapy.

PAG10

THE DORSAL SKINFOLD CHAMBER (DSC): A NEW MODEL TO STUDY PROSTATE CANCER METASTASIS IN VIVO

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Cancer metastasis to the skeleton is a major problem with prostate cancer. Intervention to prevent metastasis would be the most significant advance for the majority of patients. The aim of this work is to establish an *in vivo* model of prostate cancer metastasis.
Metatarsals (II-IV) from newborn mice are engrafted into a DSC implanted on an adult SCID mouse (6-7 weeks old). Metatarsals are rapidly re-vascularised by inosculation with the host vasculature by day 5-7. Following vascularisation fluorescently labelled (GFP) prostate (PC3), breast (MDA-B02) or oral (SCC4) cancer cells (1 x 10^5) are injected via the heart to simulate micrometastatic spread. Recordings of the DSC/metatarsal are made at 24 hr intervals for 3 weeks to identify fluorescent tumour cells. Tumour cells have been detected in the metatarsal area within 5 minutes of intra-cardiac injection. These cells are thought to be initially adhering to microvascular endothelium and/or to the metatarsal matrix 7 days after injection (10 ± 7). DSC containing the metatarsal is removed at varies time points and fixed for immunohistochemistry and bone morphology studies: including CD31 and CD105.
This model will allow homing, interactions and extravasation between the tumour and bone marrow cell populations to be studied in detail. Funded by Sixth European Framework Programme (PROMET).

PAG11

COLLATERALIZATION UPON MICROVASCULAR OCCLUSION: A SIMULATION APPROACH

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Terminal vascular beds continually adapt to hemodynamic and metabolic conditions. Previously, a model simulation was established, which allows to simulate realistic adaptation of vessel diameters in microvascular networks: all vessels change their diameter in response to calculated wall shear stress, transmural pressure and a metabolic stimulus, acting locally and up- and downstream.
Here, this approach was used to investigate responses to simulated occlusion of a major arteriole using data for rat mesentery microvascular networks. Changes in flow pathways and tissue PO2 resulting from occlusion and subsequent diameter adaptation were analyzed.
Upon occlusion, initially hypoxic tissue regions (PO2 < 1 mmHg) regained an adequate oxygen level due to formation of collateral flow pathways (post- / pre-occlusion PO2 = 1.05, Median). Initial data indicate, that this collateralization process occurs primarily in response to metabolic signals conducted upstream from underperfused tissue regions and to local shear stress. Collateralization includes transformation of capillaries and venules to larger arterioles and partial flow reversal. Thus, adaptation rules derived from steady state diameter distributions seem to be capable of evoking effective collateralization after vessel occlusion guaranteeing an adequate tissue oxygenation.

PAG12

PATENT DUCTUS ARTERIOSUS HAS PROFOUND EFFECTS ON THE MICROCIRCULATION IN PRETERM INFANTS

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The functional vessel density (FVD) and vessel diameters were measured with Sidestream Dark Field Imaging (SDF) in 15 preterm infants (mean birth weight: 845g [95% CI: 686-1004g]) with hemodynamically significant patent ductus arteriosus (PDA) and in 12 preterm infants (mean birth weight: 940g [95% CI: 828-1053g]) without PDA (C). Measurements started on the third day of life until medical closure of the PDA respectively until day 8 in the control group. Images were blinded and analyzed with Microvision Analysis Software (MAS).
Prior to PDA treatment FVD was significantly lower (PDA mean: 260 [95% CI: 245 - 274] vs. C: 295 [277 - 313] cm^{-1}, p=0.003) with less large vessels (diameter > 20µm: PDA: 6% [4% - 8%] vs. C: 12% [6% - 17%], p=0.04) and more small vessels (diameter < 10µm: PDA: 43% [36% - 49%] vs. C: 30% [23% - 38%], p=0.01). After PDA closure FVD and vessel diameters did not differ between the groups. There was an inverse linear correlation between the hemodynamic significance of the PDA as measured by echocardiography and the FVD (p<0.0007).

We have shown for the first time that a PDA changes microcirculation with significant alterations in vessel density and diameters.

PAG13

BEVACIZUMAB DOES NOT PREVENT VEGF BINDING TO NEUROPILINS – AN ESCAPE MECHANISM DURING BREAST CANCER TREATMENT?

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Bevacizumab (Bz), which increases progression free survival in breast cancer patients in combination with chemotherapy, acts by blocking the VEGF-R1/R2 binding site of VEGF. However neuropilins, alternative VEGF receptors on endothelial cells, bind to a different region of VEGF. Therefore this study will test the hypothesis that Bz does not prevent VEGF binding to neuropilin (Np) and that simultaneously blocking VEGF-Nrp binding will enhance Bz treatment efficacy. Western blot analysis reveal that Nrp1 and Nrp2 are expressed in both human breast cancer cell-lines and endothelial cells. Solid phase assays show that VEGF binding to recombinant Nrp1 or MDA-MB-436 cells is not inhibited by Bz, but is inhibited by anti-Nrp1 antibody. VEGF binding to HuDMECs is inhibited by both Bz (~36%) and anti-Nrp1 antibody (~27%), whereas a combination of both antibodies results in the greatest inhibition (~97%). Moreover, both Bz and anti-Nrp1 antibody significantly ($P<0.001$) inhibit VEGF stimulated cord branch-point formation (VEGF: 43 ± 6; Bz: 25 ± 3; anti-Nrp1; 26 ± 4/field of view), whereas the combination of Bz and anti-Nrp1 caused a further reduction (17 ± 3; $P<0.003$)).

These data show that Bz does not prevent VEGF binding to neuropilins, suggesting that Bz treatment efficacy may be enhanced by use of a neuropilin blocking agent. Supported by Yorkshire Cancer Research

PAG14

EFFECTS OF STANNIOCALCIN 2 ON VESSEL FORMATION IN HUMAN NEUROBLASTOMA

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Neuroblastoma (NB) is the most frequent solid extracranial tumor in children. Despite aggressive multimodal therapy, mortality remains high in advanced stages. We recently showed that keratoepithelin-overexpression inhibits NB growth. Analysis of these cells revealed enhanced expression of stanniocalcin 2 (STC2), a secreted phosphoprotein recently discovered in human and mammals. Stc2 is expressed in various embryonic and adult tissues, and in several tumor cell lines. STC2 is associated with a favourable prognosis in estrogen receptor-positive breast cancer and its expression in mice results in pre- and postnatal growth restriction. Inhibition of STC2 renders mouse NB cells more vulnerable to apoptotic stimuli. STC2 was also found during the invasive process of blastocyst implantation in rats. To elucidate the function of STC2 during progression and vascularization of NB, we analysed a large panel of cell lines and primary tumors, and investigated effects of STC2 *in vitro* and *in vivo*. STC2-transfected NB cells showed inhibition of proliferation and increased apoptosis, however, they also showed increased invasiveness. In chorioallantoic-membrane assays, tumor formation was investigated, and an increased tendency to hemorrhage was found after STC2-transfection. In conclusion, STC2 inhibits growth of NB cells *in vivo* and *in vitro*, but makes the cells more invasive and destructive.

PAG15

QUANTITATION OF ANGIOGENESIS INHIBITION IN THE CHICK'S CHORIOALLANTOIC MEMBRANE MODEL USING AN IMAGE PROCESSING METHOD

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The chick's embryo chorioallantoic membrane (CAM) is widely used as an *in vivo* vascularization model. The dense vascular network of its respiratory membrane can be used to study angiogenic and anti-angiogenic stimuli. After topical deposition of bevacizumab (Avastin®, an anti-Vascular Endothelial Growth Factor (anti-VEGF) agent based on a humanized anti-body protein) on the CAM, this drug's activity was assessed in terms of inhibition, maturation or deprivation of the vasculature network. The aim of this study was to develop a quantitative method to characterize the vascular network of the CAM, and to compare performance of this method to that of a visual grading assessment. On the basis of fluorescence images the following descriptors were computed: the fractal dimension, the number of vascular segments/mm^2, the number of branching points/mm^2, the mean segment length, the total length of the vessel network and the number of meshes. The visual assessment of the vasculature was performed based on the number, the size of the avascular zones, and on the 'ring like' aspect of the capillaries. We showed that the quantitative evaluation of descriptors of the vascular network is reliable and correlated with the somewhat more subjective visual grading of this vasculature.

Tuesday, August 26th
Vascular remodelling
Poster Session - PVR

PVR1

SMOKING INDUCED PULMONARY VASCULAR REMODELING PRIMARILY AFFECTS DISTAL MICROCIRCULATION

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Early stages of chronic obstructive pulmonary disease are characterized by the remodeling of lung vasculature which is primarily triggered by the pulmonary endothelial dysfunction. The latter process may be either directly induced by the smoke components, or as a consequence of the inflammatory reaction. Accordingly, the aim of our study was to characterize the smoking induced structural abnormalities of pulmonary circulation. In tissue sections derived from fragments of pulmonary exeresis (smokers n = 10, non-smokers n = 5) we have performed the morphometric and immunohistochemical examinations of pulmonary arteries. Our results showed in smokers a process of arteriolar muscularization, intimal thickening (intima index of 24.37 3.16 in smokers, vs. 17.86 2.96 in controls, p = 0.002) and an endothelial tumefaction (suggestive for impairment of endothelial cells with subsequent hyperpermeabilization). The process of intimal thickening was correlated with cigarettes consumption expressed as pack-year (Pearson coefficient was r = 0.65, p = 0.04) and was found to affect mainly pulmonary arteries with small diameter (r = –0.59, p = 0.02 for the association intima index - vascular diameter). In conclusion, our data suggest that pulmonary arterioles represent the vascular territory mainly affected by smoking induced structural abnormalities.

PVR2

INDUCTION OF CEREBRAL ARTERIOGENESIS LEADS TO EARLY-PHASE EXPRESSION OF PROTEASE INHIBITORS IN GROWING COLLATERALS OF THE BRAIN

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Cerebral arteriogenesis constitutes a promising therapeutic concept for cerebrovascular ischemia, however transcriptional profiles important for therapeutic target identification have not yet been investigated. This study aims at a comprehensive characterization of transcriptional and morphological activation during early-phase collateral vessel growth in a rat model of adaptive cerebral arteriogenesis.

Arteriogenesis was induced using a 3-vessel occlusion (3-VO) rat model of non-ischemic cerebral hypoperfusion. Collateral tissue from the growing posterior cerebral artery (PCA) was selectively isolated avoiding contamination with adjacent tissue.

We detected differential gene expression 24h post 3-VO with 164 genes significantly deregulated. Expression patterns contain gene transcripts predominantly involved in proliferation, inflammation and migration. A significant number of genes deregulated within the proliferating collateral contain shear stress responsive elements and/or NF-kB binding sites within their promoter regions.

Analysis of molecular annotations and networks associated with differentially expressed genes revealed that early-phase cerebral arteriogenesis is characterized by protease inhibitor expression. These results were confirmed by qRT-PCR and in-situ hybridisation localized expression of TIMP-1 and kininogen to collateral arteries, showing that TIMP-1 and kininogen might be molecular markers for early-phase cerebral arteriogenesis.

PVR3

ACE-INHIBITING WHEY PEPTIDES LOWER BLOOD PRESSURE AND INDUCE CARDIAC REMODELLING

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Specific whey peptides exert profound ACE–inhibiting activity in vitro. We now tested in spontaneously hypertensive rats (SHR) whether food augmentation lowers arterial blood pressure (BP) and results in myocardial remodelling. SHR received food pellets augmented with either an ACE-inhibiting peptide (IP), a control peptide (CP, each 1.5 mg/kg/day) or captopril (CA, 70 mg/kg/day) for 14 weeks. BP was monitored noninvasively throughout the study (tail-cuff). At week 14 coronary dilator reserve was determined (adenosine 1 µM, reactive hyperemia) in isolated heart preparations. At start of the feeding period systolic BP was similar in all groups (220-230 mmHg) and remained elevated in CP (- 3 mmHg, n.s.), whereas it was

significantly lower in IP (-18 mmHg) and CA (-25 mmHg). At week 14 heart mass was lower in IP (92 ± 1 %) and CA (83 ± 2 %, both $p < 0.05$ vs CP). Coronary flow reserve was improved in CA and IP versus CP.
In summary, functionally effective nutrition with respect to BP is feasible with specific whey peptides. The effect is similar to that of established ACE-inhibitors. BP lowering is associated with myocardial remodelling.

PVR4

MICRO-PIV AS A RESEARCH TOOL FOR IN VIVO STUDIES OF VASCULAR REMODELLING IN CHICK EMBRYOS

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We present measurements in the vitelline network of a chick embryo, an accessible model system for the study of human cardiovascular processes, by means of microscopic Particle Image Velocimetry. Small fluorescent markers, bio-inert due to a poly-ethylene-glycol coating, are injected in a vein to visualize the blood flow; these markers are rapidly distributed throughout the complete cardiovascular system and do not adhere to the lumen wall. Using local cross-correlation, the local plasma velocity can be determined with a greater accuracy and robustness than by e.g. tracking of blood cells. The results show that detailed local blood velocity measurements can be obtained, with a spatial resolution of typically 12 micrometer and a field-of-view of approximately $1x1mm^2$. Using this velocity data, the location of the vessel walls, perfusion rates and the local wall shear stress can be derived. We show that the method can be extended to reconstruct the temporal behaviour in periodic flows. Furthermore, the full three-dimensional blood velocity field can be obtained by scanning the measurement volume. The experiment can be repeated in the same embryo over the course of hours, so that developmental changes or adaptation to stimuli can be observed.

Tuesday, August 26th
Atherosclerosis
Poster Session - PAS

PAS1

SPHINGOSINE-1-PHOSPHATE AND ATHEROSCLEROSIS

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Atherosclerosis is a systemic inflammatory disease characterized by the accumulation of leukocytes, promoting atherosclerotic lesion formation. Sphingosine 1-phosphate (S1P) regulates immune cell migration acting through five G protein-coupled receptors named $S1P_{1-5}$. Here, we treated $ApoE^{-/-}$ mice with the S1P receptor "superagonist" FTY720 to define the role of S1P and S1P receptors during atherosclerosis.
We used $ApoE^{-/-}$ mice, fed with a high-cholesterol diet for 10 weeks. The animals were treated for 10 weeks with FTY720 (n= 8) or vehicle (DMSO, n=8). Intravital microscopy was performed to monitor leukocyte adhesion dynamics in the process of atherosclerosis. For quantitative assessment of the plaque area mice were sacrificed, the carotid artery and the aortic arch were dissected free and stained with Sudan III.
The number of firmly adherent leukocytes in the common carotid artery and in the carotid bifurcation was dramatically reduced by FTY720 application compared to vehicle (by 79% and 89%, respectively). Furthermore, FTY720 reduced the plaque area in the aortic arch and the common carotid artery when compared with vehicle (by 43% and 13%, respectively).
Together, our results show that FTY720 inhibits the development of atherosclerosis in mice. Pharmacological suppression of leukocyte accumulation within the vascular intima by FTY720 is an effective approach to attenuate plaque formation.

PAS2

ENDOTHELIAL CELLS AT ATHEROPRONE SITES EXPRESS ACTIVATED C-JUN N-TERMINAL KINASE (JNK) WHICH INDUCES PRO-APOPTOTIC TRANSCRIPTS

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The greater curvature of the aorta is exposed to high shear and is protected from endothelial cell (EC) apoptosis, inflammation and atherosclerosis, whereas the lesser curvature exposed to low shear is atheroprone. Pro-inflammatory mediators (e.g. LPS, TNF□) activate JNK MAP kinase by phosphorylation. We studied the interaction between shear stress and LPS *in vivo* by *en face* staining and showed that phosphorylation of JNK in EC occurs constitutively in the lesser curvature of the aorta of C57BL/6 mice and can be enhanced by LPS treatment ($p<0.001$ vs. untreated). In contrast, JNK activation was suppressed in the greater curvature of untreated or LPS-treated mice ($p<0.05$ vs. lesser curvature). The physiological role of JNK in cultured EC was examined using a specific pharmacological inhibitor (CT536706). Transcriptional responses to pro-inflammatory mediators were assessed either in the presence or in absence of CT536706 using Affymetrix® microarrays followed by functional annotation using Rosetta-resolver® software. TNF□ (10ng/ml, 4h) induced 165 transcripts including regulators of inflammation (n=34) and apoptosis (n=10). JNK was necessary for the induction by TNF□ of most pro-inflammatory (29/34) and pro-apoptotic (9/10) transcripts. We conclude that JNK activation in EC at atherosusceptible sites may contribute to atherosclerosis by inducing pro-apoptotic as well as pro-inflammatory molecules. Supported by the British Heart Foundation and the National Heart and Lung Institute Foundation, UK.

PAS3

NON-SPECIFIC ENDOCYTOSIS AND CHOLESTEROL CRYSTALS AS A CAUSE OF ENDOTHELIAL INJURY OF MESENTERIAL VESSELS IN RATS.

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Objective: According to M.M. Ovchinnikov's theory, non-specific endocytosis of cholesterol crystals inside the endothelial cells in small mesenterial vessels makes these cells porous and then injured. This process mediates immediate type of hypersensitivity, which forms an immunodeficiency secondary to atherosclerosis cased by small vessels endothelial layer injury. Methods: Lyposomes with LDL and HDL were infused using cannula into the blood flow of mesenterial arteries of rat males from 1 to 2 years of age. Scavenger receptors, class B, type I (SR-BI), which control low density lipoprotein (LDL), were blocked by monoclonal antibodies against native human SR-B1. Rats were sacrified, mesenterial arteries endothelium studied for size and shape of cholesterol crystals. Splenocytes isolation, phenotyping of CD4+ and CD25+ performed. Concentration of proinflammatory cytokines IL-2, Interferon-□ and TNF-α in blood have been studied. Results. Ruts with increased LDL level demonstrated many sings of immunodeficiency. The IL-2, TNF -α levels were increased in intact animals. Interferon-□remain the same in scavenger blocked and intact rats. The CD4+ quantity was increased in intact animals in comparison with scavenger blocked ones. Conclusion: Nonspecific endocytosis of cholesterol crystals involves immune mechanisms in ruts. Next step of research will include the immune pharmacological correction of scavenger receptors interactions.

PAS4

IMPAIRED MICROVASCULAR REACTIVITY PREDICTS ATHEROSCLEROTIC DISEASE

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Our study was performed to examine whether microvascular reactivity-derived parameters are associated with cardiovascular risk factors/events and predict atherosclerotic disease.
Microvascular reactivity was determined as the effect of postocclusive reactive hyperemia (PORH) and local heating (LH) measured with laser Doppler flowmetry at forearm skin in 105 subjects, with (85) and without (20) cardiovascular comorbidities. Parameters studied were maximum percentage of PORH response ($PORH_{\%}$), maximum increase in hyperemic perfusion ($PORH_{max}$), time to peak (Tp), mean velocity of the hyperemia increase ($PORH_{max/Tp}$), area under the curve (AUC) of the hyperemia and maximum percentage of LH induced hyperemic response ($LH_{\%}$).
Subjects with increased number of cardiovascular risk factors/events had decreased $PORH_{\%}$, $PORH_{max}$, AUC and $LH_{\%}$. Subjects were classified by tertile according to the values of microvascular reactivity. Subjects in the worst tertiles of $PORH_{\%}$, $PORH_{max}$, $PORH_{max/Tp}$, AUC and $LH_{\%}$ had markedly higher prevalence of atherosclerotic disease. After adjustment for age, male gender, body mass index, hypertension, diabetes mellitus and hyperlipidemia, the worst tertiles of $PORH_{\%}$, $PORH_{max}$, $PORH_{max/Tp}$ and AUC were independent predictors of atherosclerotic disease (adjusted OR: 17.94; 6.31; 4.09 and 12.75, respectively).
These findings suggest that lower $PORH_{\%}$, $PORH_{max}$ and AUC parameters are associated with cardiovascular disease and predict atherosclerotic event.

PAS5

SELECTIVE PHOSPHODIESTERASE-5 INHIBITION REDUCES NEOINTIMA FORMATION IN RAT CAROTID ARTERIES AFTER ENDOTHELIAL DENUDATION

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Long-term results of successful vessel reconstruction are compromised by restenosis due to neointima formation. Recent studies suggest that nitric oxide (NO) may play a role in the reduction of neointima formation. The aim of our study was to evaluate the effectiveness of the NO-cGMP pathway by administering a selective phosphodiesterase-5 inhibitor Vardenafil (V) in a rat model of carotid stenosis. Carotid endarterectomy was performed on male Sprague-Dawley rats. After cross clamping, the intima was denudated and the arteriotomy was closed. Three groups were studied: sham-operated (n=6), control endarterectomized (E,n=6) and endarterectomized rats treated orally with Vardenafil (E+V,10mg/kg/day,n=6). After 3 weeks, the vasculatures were perfusion-fixed (formaldehyde,4%,30ml/min). Vessel compartment areas were measured by microscopy using HE staining. Neointima areas, stenosis grade and neointima/media area ratio were compared between groups. Immunohistochemical analysis was performed to confirm neointima formation (TFG-$beta_1$,alfa-sm-actin), apoptosis (TUNEL-staining) and local cGMP-content. cGMP plasma levels were determined by enzymeimmunoassay. Three weeks after endarterectomy, marked neointimal hyperplasia was found in the control group. Phosphodiesterase-5 inhibition significantly reduced the stenosis grade (20.42+/-7.42% vs. 53.69+/-12.07%) and neointima/media area ratio (0.51+/-0.18 vs. 1.08+/-0.21). We revealed higher plasma cGMP-levels in the treatment group. Phosphodiesterase-5 inhibition suppressed neointimal hyperplasia in a rat model of carotid stenosis.

PAS6

THE ROLE OF PLATELET-INDUCED VASCULAR PROGRANULIN EXPRESSION IN ATHEROPROGRESSION

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Platelets are thought to play a crucial role in the inflammatory process of atherosclerosis. However, the molecular determinants of platelet-induced vascular inflammation remain to be elucidated. Here, we defined the platelet-mediated alterations in vascular gene expression patterns in the ApoE-deficient ($ApoE^{-/-}$) mouse model. In $ApoE^{-/-}$-mice platelet adhesion was chronically inhibited by repetitive i.p. administration of a monoclonal anti-GPIbα antibody. $ApoE^{-/-}$-mice treated with an irrelevant isotype antibody and Wt-mice (C57Bl6/J) served as controls. In all groups, gene expression analysis of atherosclerotic lesion prone sites of the aortic arch was carried out. Notably, the expression of progranulin (PGRN) was significantly upregulated in atherosclerotic lesions of $ApoE^{-/-}$-mice treated with isotype control antibodies, whereas in anti-GPIbα-treated animals PGRN expression was similar to healthy Wt control mice. In situ-hybridisation revealed that PGRN expression was restricted to areas of atherosclerotic plaques. To determine the functional role of PGRN in cell proliferation, HUVECs were cultivated in the presence of soluble murine PRGN-Fc peptides or control Fc-peptides. Both, endothelial cell proliferation as well as migration towards an artificial scar in the endothelial monolayer was significantly increased in the presence of mPRGN-Fc. This supports a crucial role of PGRN in the regulation of endothelial cell proliferation and migration. Next, the effect of PGRN on atheroprogression was assessed *in vivo*. $ApoE^{-/-}$-mice were chronically treated with mPRGN-Fc peptides or control Fc-peptides (n=5 each group). After 12wks the aortic arches were harvested, stained with Sudan red and

the level of atherosclerotic lesion formation assessed using en-face quantification. Interestingly, chronic treatment with PRGN-Fc substantially accelerated atherosclerotic lesion formation. Together this indicates that platelet adhesion triggers PGRN expression in the atherosclerotic vessel wall. Enhanced expression of PGRN in turn appears to play a crucial role in the manifestation and progression of atherosclerotic vascular lesions.

PAS7

ACTIVATION OF MTOR KINASE BY CXCR3 CHEMOKINES IP10 AND MIG PLAYS A CRUCIAL ROLE IN CARDIOVASCULAR DISEASE

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Atherosclerosis, restenosis and post-transplant graft atherosclerosis are characterized by intimal hyperplasia as a result of endothelial damage, infiltration of leukocytes, recruitment of hematopoietic progenitor cells and accumulation of smooth muscle cells. The CXCR3 chemokines IP10 and MIG have been implicated in vascular repair; however, the molecular mechanisms affected hereby remain elusive. Using genetic and pharmacological CXCR3 deletion in a mouse model of arterial injury we show for the first time that the IP10/MIG/CXCR3 axis is critical for injury-induced mTORC1 activation governing subsequent inflammation, apoptosis, recruitment of progenitor cells, re-endothelialization and intimal hyperplasia. Vascular injury induced IP10 and MIG mRNA and protein expression resulting in recruitment of $CXCR3^+$ cells and intimal hyperplasia. Moreover, wire injury induced profound upregulation and activation of mTORC1 leading to an increased phosphorylation of its downstream target p70 S6 kinase. Unexpectedly, inhibition of the CXCR3 axis abolished mTORC1 activation resulting in a diminished apoptosis of intimal cells and reduced recruitment of inflammtory and hematopoetic progenitor cells after vascular injury. Thereby, deletion of the CXCR3 axis yielded a significant reduction of intimal hyperplasia.
These findings establish the CXCR3 axis as a major player in mTORC1 activation during vascular remodeling, a process, which is essential for vascular repair.

Tuesday, August 26th
Clinical assessment of microvascular function
Poster Session - PCA

PCA1

ARTERIAL STIFFNESS AND MICROVASCULAR REACTIVITY IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Background: Hypercholesterolemia is common in primary biliary cirrhosis (PBC) due to chronic cholestasis. However, no increased cardiovascular risk was reported previously. Endothelial dysfunction and arterial stiffness are early markers of atherosclerosis. Therefore we investigated arterial stiffness and endothelial function in PBC.
Methods: In 26 PBC-patients (age:65±12yrs, females, cholesterol: 6.1 1.0 mmol/l) and in age-matched hypercholesterolemic subjects (n=22, age:64±11yrs, females, cholesterol: 5.8 1.0 mmol/l) arterial stiffness was evaluated by measurement of carotid-femoral pulse wave velocity (PWV). Skin microvascular reactivity was measured by laser Doppler flowmetry in the forearm during post-occlusive reactive hyperaemia (PORH), local heating and administration of vasodilators (acetylcholine/Ach, sodium nitroprusside/SNP, respectively) by iontophoresis.
Results: Percent changes in peak flow during both acetylcholine (Ach) and sodium nitroprusside (SNP) administration were higher in PBC patients (371.6±246.3% vs. 236.4±193.6%, p=0.03 and 683.6±882.9% vs. 441.8±323.5%, p=0.02). Vasodilatation (%) during PORH was also higher in PBC patients (PF%: 456.8±184 vs. 340.5±146.6 PU, p=0.01). There was no difference between the two groups either in the PWV (10.9±2.8 m/s vs. 10.85±2.5m/s, p=0.39) values or in response to local hyperthermia.
Conclusion: Our data on for "defending" mechanisms against hypercholesterolemia and consequently against increased cardiovascular risk in PBC.

PCA2

PLASMA LOW DENSITY LIPOPROTEIN-CHOLESTEROL (LDL-C) WITHIN THE NORMAL RANGE PREDICTS LARGE ARTERY COMPLIANCE

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We tested the hypothesis that plasma LDL-C concentration below that considered clinically 'high' (<4.1 mmol/L; NCEP guidelines) was a significant predictor of large artery compliance (CC; via carotid ultrasound and tonometry) in a cross-section of 115 carefully screened, healthy, non-smoking, un-medicated men with a broad age range. LDL-C was significantly associated with CC (r=-0.36, p<0.001). We then used regression and part-correlation (part-r) to determine the association of LDL-C with CC independently of age, aerobic fitness (maximal oxygen uptake) and waist circumference. Both independent variables predicted CC in models with LDL-C and age (part-r=-0.21, p=0.02 and part-r=-0.36, p<0.001, respectively), LDL-C and aerobic fitness (part-r=-0.21, p=0.02 and part-r=0.42, p=0.001), but only LDL-C predicted CC in the model with LDL-C and waist circumference (part-r=-0.24, p=0.02). In a combined model with all four independent variables, LDL-C remained a significant predictor of CC (part-r=-0.18, p=0.04). Our analyses indicate that LDL-C below that considered clinically high predicts directly measured CC, and that this is independent of age, aerobic fitness and waist circumference. The possibility that LDL-C within this range affects vascular health warrants further investigation. This could have relevance to the primary prevention of diseases associated with large artery stiffening.

PCA3

EVALUATION OF MICROVASCULAR ENDOTHELIAL FUNCTION IN PATIENTS WITH TYPE 1 DIABETES USING LASER-DOPPLER PERFUSION MONITORING: WHICH METHOD TO CHOOSE?

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We compared different methodologies in the assessment of skin microvascular endothelial function in patients with type 1 diabetes. This cross-sectional study included 50 outpatients with type 1 diabetes and 46 control subjects. Skin microvascular reactivity was assessed using laser-Doppler perfusion monitoring coupled with different local physiological (post-occlusive reactive and thermal hyperemia) or pharmacological (dose-response curves for iontophoresis of acetylcholine and sodium nitroprusside) stimuli. Microvascular vasodilator responses to both acetylcholine (P<0.01) and sodium nitroprusside (P<0.05) were significantly reduced in patients. Conversely, increases in microvascular flow induced by post-occlusive (P>0.05) and thermal (P>0.05) hyperemia were not significantly different between patients and controls. In conclusion, our study suggested that different methodologies designed to assess skin microvascular endothelial function generate dissimilar results in patients with type 1 diabetes. Thus, the use of a single physiological or pharmacological stimulation coupled to laser-Doppler perfusion monitoring should be avoided. Supported by FAPERJ and CNPq.

PCA4

EFFECTS OF ANGIOTENSIN II INFUSION ON INSULIN-INDUCED MICROVASCULAR FUNCTION, BLOOD PRESSURE REGULATION, AND INSULIN SENSITIVITY IN HEALTHY SUBJECTS.

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Insulin induces vasodilatation and capillary recruitment, thereby increasing whole body glucose uptake (WBGU), and decreasing blood pressure. Angiotensin II (AngII) has been shown to enhance insulin-stimulated WBGU. However, the mechanism of this action is unclear. The aim of study was to determine the effects of AngII on insulin-induced microvascular function, insulin sensitivity, and blood pressure in healthy subjects.
Methods: we studied the effects of AngII or phenylephrine (PE) infusion on skin perfusion and endothelium-(in)dependent reactivity (laser-Doppler flowmetry and iontophoresis), WBGU, and blood pressure in 12 lean normotensive subjects during a hyperinsulinaemic euglycaemic clamp ($50mU \cdot kg^{-1} \cdot hr^{-1}$).
Results: Insulin enhanced the acetylcholine-induced increase in skin perfusion ($p<0.012$). Infusion of PE, and not AngII, augmented this insulin-mediated effect ($p=0.04$). WBGU increased similarly with AngII and PE. AngII and PE had an equal pressor response without the clamp, but during hyperinsulinaemia the pressor response to PE, but not to AngII, was reduced ($p=0.01$).
Conclusion: insulin induces microvascular dilatation possibly via the endothelium. This effect is not augmented by AngII, suggesting no interaction between insulin and AngII signaling pathways at the level of small arterioles/venules. Our blood pressure data, however, do suggest such an interaction at the level of the resistance arteri(ol)es. Hence, possible interactions of microvascular effects of insulin and AngII seem to vary within the levels of the microcirculation.

PCA5

THE ENDO-PAT2000 AND FULL-FIELD LASER PERFUSION IMAGER FOR MEASURING VASCULAR FUNCTION: RESULTS FROM A REPRODUCIBILITY STUDY

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Peripheral arterial tonometry (PAT) and full-field laser perfusion imaging (FLPI) are new tools used to non-invasively study vascular function. We evaluated the reproducibility of both devices in 20 males, divided in two age groups (I: 20-40 years; II: 40-60 years). Fingertip PAT allowed to calculate reactive hyperemia index (RHI) and arterial stiffness (AI). FLPI signals, expressed as flux, were simultaneously recorded. Mean and variance were calculated from 3 different recording days (days 1, 2 and 8) in order to determine within-and between-subject coefficient of variance (CV). Whereas RHI values between the groups did not differ (I: 1.70±0.42; II: 1.85±0.5), AI values were significantly different (I: -2.35 ± 12.8; II: 8.74 ± 11.8; p=0.001). Within- and between-subject CV for the RHI and AI response were 20% and 26%, and 145% and 407%, respectively. FLPI-measured flux at baseline was similar for both groups (I: 69±21; II: 70±21). Cuff inflation significantly decreased blood flow associated with low flux values (I: 22±7; II: 21±5) but rapid cuff deflation increased flux by 300% vs baseline (I: 267±58; II: 273±83). Within- and between-subject CV for the FLPI response were around 20%. Both techniques show an acceptable degree of reproducibility. Moreover, PAT measured arterial stiffness was significantly increased in older subjects.

PCA6

PHYSIOLOGICAL MEASUREMENT OF CENTRAL VASOCONSTRICTIVE TONE AND LOCAL CUTANEOUS VASOCONSTRICTIVE SENSITIVITY FOR MODELING PURPOSES

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Currently many thermoregulatory models incorporate cutaneous perfusion, as it plays a major role in heat transport. The magnitude of perfusion is generally described as a function of temperature. Some physiological models, such as formulated by Stolwijk, Tanabe and Fiala, incorporate central tonus and local sensitivity explicitly. However, used perfusion control parameters have been estimated and are not based on physiological measurements. Therefore, their applicability might not be justified. In this research a method is proposed to measure central sympathetic vasoconstrictor tone and local cutaneous vasoconstrictor sensitivity. Together, the protocol was performed on males and females, young adults (18-28yr) and elderly (68-78yr) and on both glabrous and non-glabrous skin. Local cutaneous perfusion is measured by laser-Doppler flowmetry. Through iontophoresis of Bretylium tosylate (10mM) sympathetic nerve terminals are blocked, such that local endogenous release of sympathetic neurotransmitters is abolished. Afterwards a superphysiological dose noradrenaline (2.5mM) is administered through iontophoresis. The reduction in perfusion is used as a measure for vasoconstrictor sensitivity at maximal vasoconstrictor tone. This is repeated over a range of local temperatures. Then the whole body is cooled and the decrease in perfusion at a non-bretylium site is measured. Combining perfusion and local sensitivity, vasoconstrictor tone is described as a function of temperature.

PCA7

MICROCIRCULATION DISORDERS UNDER CHRONIC VENOUS INSUFFICIENCY: DIAGNOSTICS AND CORRECTION WITH TAXIFOLIN

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The aim of the study was to evaluate the influence bioflavonoid Taxipholin [Dihydroqercetin], possessing antioxydative action, on a skin microcirculation at patients with chronic venous insufficiency [CVI]. 45 patients with CVI [CEAP stage 2 - 4] have been surveyed at treatment of 0.3 % gel Taxipholin in comparison with treatment of 2 % gel Troxerutine [control group]. Application of gel was made on a leg and foot daily within 3 weeks. *Methods.* The estimation of skin microcirculation in a leg and foot was made by means of Laser Doppler flowmetry [LDF], and also TV-computer capillaroscopy of eponichii on 1 finger foots. *Results.* In 100 % of patients with CVI disorders of microcirculation have been revealed. Under biomicroscopy structural changes in microvessels, rheological shifts and impairment of permeability in walls have been found. The degree of microcirculation insufficiency directly depends on CVI severity. The leading pathogenetic mechanisms in microcirculation disorders are: progressive increase of the venous stagnation in microvessels, local blockage of capillary blood flow and sharp damage of barrier function in microvessel walls. The most vulnerable is the nutritive part of the microvascular network. Blood flow disorders in CVI lead to changes in myogenic activity in arterioles and precapillaries. At LDF reduction of fluxmotion amplitude, spectral narrowing of oscillations and displacement of their dominant to the high-frequency area have been revealed. We consider that it is the result of vasomotor mechanism suppression. Application of gel Taxipholin positively affects dynamics of parameters of a local and systemic microcirculation at patients CVI. After the lead treatment improvement of microcirculation and diminish of stagnation tissue blood flow was observed: average linear speed of erythrocytes in capillaries increased with 320 ± 5,4 μm/c up to 358 ± 8,1 μm/c, the degree of erythrocyte aggregation and adhesions of leukocytes decreased, the parity of arteriolar and venular diameters was normalized, the perivascular zone [on 20 - 25 %] decreased. *Conclusion.* Taxipholin possesses vasotropic action at the level of microvessels and owing to antioxidant, action positively influences on normalization of microcirculation and decrease in permeability of capillary wall at patients with CVI.

PCA8

SKIN MICROVASCULAR FLOW SPECTRAL ANALYSIS IN DIABETES AND ITS CONNECTION TO CARDIOVASCULAR NEUROPATHY

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Cardiovascular neuropathy is common complication of diabetes mellitus, brings higher morbidity and mortality due to sudden cardiac death. Some literary data showed there is no correlation between cardiovascular autonomic neuropathy (CAN) and skin microcirculation flow. Whereas in many studies is decreased sympathetic activity supposed to be a reason of constricted nutrition capillaries in favour of arteriovenous shunts. Our task was to find relation between cardiovascular neuropathy and spectral power of skin microvascular flow variability (vasomotion). In 45 patients with diabetes mellitus type 1 was examined CAN by standard methods (Ewing tests, spectral analysis) and foot skin microcirculation by laser-doppler flowmetry with spectral power calculations in ranges corresponding with endothelial, sympathetic, intrinsic myogenic, breathing and cardiac activity. Subjects were divided into two groups with and without CAN; groups were comparable in age, gender, diabetes duration, triglyceride and cholesterol level, blood pressure and BMI. There was neither observed difference in basal microcirculation flow nor in heating test (increase 8,6x vs. 8,3x). In subjects with CAN was significantly lower spectral power in endothelial ($p<0.01$) and sympathetic ($p<0.005$) range. In myogenic range was observed lower spectral power with borderline significance ($p= 0.06$) in subjects with CAN. Study was supported by Scientific programme MSM 0021620814

PCA9

DYNAMIC OSCILLATORY CIRCUIT OF REGULATION OF CAPILLARY HEMODYNAMICS AND OXYGEN EXCHANGE

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The important role of the oscillatory circuit in the regulation of number of functioning capillaries, linear and volumetric velocity of capillary blood flow, and oxygen exchange was shown by laser Doppler flowmetry with wavelet analysis of blood flow oscillations, optic oximetry, computer capillaroscopy, and thermometry of the nail bed in 30 subjects. For the first time laser Doppler flowmetry and optic oximetry were combined in one device to measure the same blood volume (LAZMA, Russia). The number of functioning capillaries is regulated by oscillations of myogenic (0.07-0.12 Hz) and sensory peptidergic (0.047-0.069Hz) origin. The appearance of sensory oscillations, especially high-amplitude oscillations, is an adaptive neurotrophic mechanism that significantly increases the number of functioning capillaries and intensity of blood flow from arterioles to capillaries. The linear velocity of capillary blood flow depends on both the oscillatory component of the microvessels tone and changes in the dynamic component of blood pressure. The contribution of oscillations varies depending on the perfusion and skin temperature. In addition to changes in the amplitude, the frequency of vasomotions may be important. The contribution of the oscillatory circuit to control of hemodynamics and oxygenation is increased with a decrease in the skin blood flow.

PCA10

INSULIN IONTOPHORESIS: A COMPARISION OF HEXAMERIC HUMAN INSULIN AND A MONOMERIC INSULIN ANALOGUE IN HEALTHY SUBJECTS

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Insulin has an important role in the vasculature. Iontophoresis has been used to investigate the microvascular actions of hexameric insulin. With the availability of smaller, monomeric insulin analogues, which are more efficient to iontophorese, it may be possible to improve insulin-mediated responses. This study aimed to compare the iontophoresis of hexameric (Actrapid) with monomeric insulin (Novorapid), in the skin microvasculature of healthy individuals.

Eight healthy subjects (4males; 22-44years) underwent cathodal iontophoresis of both monomeric and hexameric insulins, both the diluent containing insulin and the diluent alone. Cutaneous perfusion was assessed using laser Doppler fluximetry.

Actrapid and its diluent alone caused an increase in cutaneous perfusion, however, there was no difference in the response between diluent plus Actrapid and diluent alone: (Peak Response (PR):40.1(31.8-61.2) vs 51.4(31.2-74.7) perfusion units, Wilcoxon-Signed-Rank, p=0.26). Novorapid and its diluent alone increased perfusion but there was no difference between the two responses:(PR:54.3(33.2-92.3) vs 79.6(63.6-104.6) perfusion units, p=0.16). The hexameric and the monomeric insulins did not produce different perfusion responses (p=0.56).

The interpretation of the vascular responses to the iontophoresis of insulin is complicated by the large diluent alone perfusion response. This study failed to show any benefit in the iontophoresis of the smaller monomeric as opposed to hexameric forms.

PCA11

EFFECT OF CYCLOOXYGENASE AND NITRIC OXIDE SYNTHASE INHIBITION ON CUTANEOUS POSTOCCLUSIVE REACTIVE HYPEREMIA

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The aim of our study was to investigate the effect of combined endothelial nitric oxide synthase (eNOS) and cyclooxygenase (COX) inhibition on postocclusive reactive hyperemia (PRH) in the skin. In 12 males, we measured cutaneous laser Doppler flux (LDF) at the forearm. The eNOS inhibitor, L-NMMA, and the COX inhibitor, diclofenac, were applied to the forearm by an intradermal microinjection (treated site); saline was injected to a control site. Baseline LDF was measured for three minutes. Afterwards, brachial artery was occluded to suprasystolic blood pressure for three minutes. The peak LDF after the release of occlusion did not significantly differ between the control and treated sites (487+/-48% increase over baseline LDF vs. 380+/-40%, t-test). Also, the time to peak flow (7.3+/-0.9 vs. 8.9+/-1.2 seconds) as well as the recovery time (66.4+/-7.4 vs. 51.4+/-10.5 seconds) did not significantly differ (t-test) between the control and treated sites. We found a statisticaly significant difference in the area under the PRH curve between the control and treated sites (1691+/-333 PU (perfusion units)*seconds vs. 1005+/-197 PU*seconds, t-test, p = 0,028). The results imply that apart from NO and prostacyclin other factors, like endothelium-derived hyperpolarizing factor, contribute importantly to the generation of PRH in skin microcirculation.

PCA12

FEASABILITY OF SCANNING LASER DOPPLER IN ERYTHROMELALGIA

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Erythromelalgia is an uncommon disorder that manifests as red, hot, painful hands and or feet and less commonly affecting the face or trunk. It may be seen in isolation (rare) or as part of a broad complex of symptoms such as an associated with peripheral neuropathy. Etiology and pathophysiology remain unclear, but dysregulation of vasomotor tone and increased mitochondrial metabolism have both been hypothesized.

Vascular investigations in our laboratory include baseline assessment of segmental pressures, Doppler signals, temperatures and transcutaneous oximetry to rule out underlying arterial disease. The patient is studied without symptoms for baseline comparison and instructed to return to the laboratory when symptoms are present. Attempts at point laser Doppler have proven problematic in that manipulation of digits can induce symptoms.

With this background, we sought to test the feasibility of scanning laser Doppler in erythromelalgia. Scans were taken without and with symptoms as part of the vascular evaluation. Since exact locations, distances and angles cannot be ensured, a qualitative interpretation, not quantitative, was provided. The extent and change in flux change is readily apparent in the scanned image. While initial impressions from technicians and physicians are positive, further study is needed.

PCA13

THE EFFECT OF DIAZEPAM ON POSTOCCLUSIVE REACTIVE HYPERAEMIA

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We studied the effect of orally applied diazepam on the microvascular response to postocclusive reactive hyperaemia (PRH). PRH is believed to be mediated by the interaction of metabolic and myogenic mechanisms. Experiments were

performed on 9 healthy males and 11 females. Laser-Doppler (LD) flux was measured in fingertips 5 minutes before and after the release of an 8-minute digital arteries occlusion. The measurements were repeated one hour after the ingestion of diazepam. Before diazepam application average LD flux at rest (females 296.9 +/- 26.6 PU vs. males 197.2 +/- 31.6 PU) and during PRH (females 369.8 +/- 21.2 PU vs. males 292.9 +/- 18.8 PU) were significantly higher in females than in males (t-test, $p<0.05$). After the ingestion of diazepam the gender differences disappeared. The LD flux during PRH (292.9 +/-18.8 PU before vs. 357.2 +/- 41.1 PU after diazepam) and area under LD flux curve during PRH (39516 +/- 3675 before vs. 50594 +/- 5473 after diazepam) after ingestion of diazepam significantly increased only in the group of males but not in the group of females (paired t-test, $p<0.05$). Our results show that diazepam has influenced PRH LD flux response in males but not in females.

PCA14

COMPARISON OF THERMAL AND LASER DOPPLER IMAGING AS METHODS TO MEASURE RESPONSE TO COLD STIMULUS IN PATIENTS WITH SYSTEMIC SCLEROSIS AND PRIMARY RAYNAUD'S PHENOMENON AND IN HEALTHY CONTROLS

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Systemic sclerosis (SSc) is a connective tissue disease affecting microvascular function. Raynaud's phenomenon (episodic colour changes of the hands, usually in response to cold exposure) is one of its major clinical manifestations. Laser Doppler and thermal imaging are techniques used to measure skin temperature (indirectly measuring blood flow) and blood flow respectively. The aim of the study was to elucidate the relationship between these two techniques. Following acclimatisation (23 ^{o}C) and baseline imaging, 16 patients with SSc, 14 with primary (idiopathic) Raynaud's phenomenon and 16 healthy controls underwent cold stimulus. Both hands (gloved) were placed into water (15 ^{o}C, one minute). Immediately following removal, the hands were imaged (ungloved) for 15 minutes to monitor re-warming and reperfusion. Analysis of the baseline images and re-warming curves was carried out. Correlation ($p<0.001$) was found between laser Doppler and thermal imaging for baseline (0.667) and maximum (0.729) blood flow and skin temperature and for the areas under the re-warming curves (0.684). Significant differences were found between groups (ANOVA) with both techniques for all features analysed. Laser Doppler and thermal imaging give equivalent information on cutaneous microcirculation following cold stimulus and can differentiate between patient and healthy control groups. A. K. Murray is supported by a University of Manchester Stepping Stones Award. This study was funded by the British Skin Foundation (registered charity no. 313865)

PCA15

EVALUATION OF ENDOTHELIAL FUNCTION WITH NON-INVASIVE METHODS IN DIFFERENT CARDIOVASCULAR DISEASES

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Background: Assessment of microvascular function and arterial stiffness could have diagnostic and prognostic importance in different cardiovascular diseases.
Objective: The aim of our study was to evaluate microvascular reactivity and arterial stiffness with non-invasive methods in patients with different cardiovascular risk factors.
Methods: Following blood pressure measurement, where pulse pressure (PP; mmHg) was also calculated, skin microcirculation was studied with laser Doppler flowmetry (Periflux 5001). The effect of local heating (LH; 44˚C, 1 min) and the postocclusive reactive hyperemia (PORH; 220 mmHg, 3 min) was measured. Arterial stiffness was evaluated with the newly developed TensioClinic Arteriograf instrument which registers the pulse wave curve with oscillometric method and automatically calculates the pulse wave velocity (PWV, m/s) and augmentation index (Aix, %). Healthy controls (n=13), patients with essential hypertension (EH, n=13), with essential hypertension and peripheral artery disease (EH+PAD, n=22), and essential hypertension and 2-type diabetes mellitus (EH+DM, n=25) were measured.
Results: PP was higher in EH+PAD (62.66 mmHg, $p<0.05$) and EH+DM (67.65 mmHg, $p<0.001$) groups compared with controls (52.53 mmHg). Aix, PWV and the reactive hyperemia for PORH were significantly different in healthy controls (-62.11%, 7.01m/s, 393.77%, resp.) and in the patient groups. Moreover, significant differences were found in these parameters amongst the EH (-34.25%, 7.91m/s, 292.77%), EH+PAD (0.34%, 9.16m/s, 182.86%) and EH+DM groups (0.87%, 9.27m/s, 192.84%). The reactive hyperemia for LH was significantly lower in the EH (782.08%), EH+PAD (651.32%) and EH+DM (453.76%) compared with the controls (1049.08%). Significant correlation was found between the PORH and Aix ($r=-0.54$; $p<0.001$) and PP and Aix ($r=0.42$, $p<0.05$).
Conclusions: Using these non-invasive methods there is a growing possibility to diagnose endothelial dysfunction in patients with different severity of cardiovascular diseases. Prospective studies are needed to evaluate the prognostic value and the utility in therapy follow-up of these methods.

PCA16

OMENTAL MICROCIRCULATION DURING FREE OMENTAL FLAP AUTOTRANSPLANTATION IN DOGS: INTRAOPERATIVE MONITORING BY LASER DOPPLER TISSUE FLOWMETRY

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BACKGROUND: In human esophagus operation, improving surgical security of anastomoses has a great importance. Thus, in animal experiment, a prevascularization operative technique has been developed using autologous free omental flaps at the sites of the planned anastomosis. The aim of this study was to investigate if laser Doppler tissue perfusion monitoring (LDPM) can be used in order to measure and monitor the auto-transplanted free omental flap microcirculation during these animal operations. MATERIALS AND METHODS: A total of 19 operations on 12 dogs were performed in order to develop a model in which resection of the cervical part of the esophagus involves two steps. The first step comprises omental flap free transplantation from the abdomen to the cervical region by a microsurgical method. The second step is segmental resection of the esophagus 14 days later. In this study we measured the microcirculation of mapped standard points of the omental flap before and after isolation, after omentum flap transplantation, before the resection of the esophagus, after esophageal resection and on the end of the study. We used single channel laser Doppler tissue flowmeter in parallel with hemorheological and histological investigations for angiogenesis. RESULTS: Of the 5 transplanted grafts, 4 still survived the transplantation. Both 2 omental flaps measured and monitored by LDMP were survived the whole operative series. Histological results also demonstrated the presence of angiogenesis. CONCLUSION: The measurements imply that LDPM has the potential to be used as an intraoperative monitor for tissue micro-circulation during omental flap transplantation in dogs. The prevascularization procedure can improve the surgical security for esophageal anastomosis.

PCA17

THE USE OF COMPARTMENTAL MODELLING TO EVALUATE TCPO2 DISTRIBUTION RATES: INFLUENCE OF AGE

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Microcirculation assessment by Laser Doppler Flowmetry (LDF) and transcutaneous oxygen (tcpO2) has been applied to clinics having in mind that reperfusion and oxygen distribution rates may be reliable predictors of vascular impairment. After defining a new bi-compartmental model to analyze tcpO2 and LDF data from dynamical maneuvers, the authors applied this model to a group of normal individuals (young *versus* old) to evaluate any eventual influence of age on these parameters.
Sixty healthy volunteers (both gender) were distributed in two groups (I: 18-35 years; II: 40-65 years) after informed written consent. TcpO2 and blood flow was measured in the lower limb, trough a Periflux system (Perimed, Sweden) for 10 min. Two provocations were applied to each volunteer during 10 min (A: postural change; B: Reactive hyperaemia). A bi-compartmental model was fitted to each data sets using MS Excel.
Results show that the model adequately describes these data suggesting that the *disappearance - reappearance O2 rates* during provocations could be attributed to a peripheral distribution. The rate of tcpO2 distribution is slower in group II suggesting that, although in the absence of any declared health problem, some degree of vascular impairment (surely related with age) is detected by the model.

PCA18

THE INFLUENCE OF ACUTE MENTAL STRESS ON CARDIAC AND LOCAL VASCULAR ACTIVITY IN MEN AND WOMEN

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Mental stress has been linked to cardiovascular morbidity, particularly among men, but the pathogenesis has not been clarified yet. To find out the changes in cardiovascular system provoked by acute mental stress we have measured the effect of standard arithmetic challenge on autonomic nervous system, cardiac action and peripheral vascular activity in men and women. Heart rate variability, blood pressure (Finapres), baroreflex sensitivity (BRS), cardiac output (impedance plethysmography) and the cutaneous LD flux (Perimed) were measured during and after stress in 20 healthy normotensive males (23 2 years) and 18 age matched females. A spectral analysis of interbeat intervals was performed by the autoregression method. BRS was estimated with the sequence method. During mental stress, heart rate, mean blood pressure, cardiac output and sympathetic tone increased compared to relaxation phase in both groups, whereas cutaneous LD flow and BRS decreased. In our experimental settings no statistically significant differences were found between men and women except in cardiac output, where females exhibited greater cardiac output (8,0 0,6 l/min) in response to stress as compared to males (6,2 0,5 l/min) ($p<0,05$). Our results suggest that mental stress affects cardiac action and peripheral vascular activity through the sympathetic activation mainly the same in men and women.

PCA19

REPRODUCIBILITY OF LOCAL THERMAL HYPEREMIA AND POST-OCCLUSIVE REACTIVE HYPEREMIA ASSESSED WITH LASER DOPPLER FLOWMETRY IN HUMAN SKIN

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The study of microvascular function can be routinely performed in humans using laser Doppler flowmetry of the skin. Post-occlusive hyperemia and local thermal hyperemia are used as tools to investigate endothelial and neurovascular microvascular function. However, there is no consensus over their reproducibility and variation with room temperature,

prandial status, and stress. Our objective was to test the reproducibility of those techniques on the finger and on the forearm, and their physiological variations.
We performed local heating up to 44°C and 5 min postocclusive hyperemia in twelve healthy volunteers, at day 0 and day 7. We also tested the impact of a standard meal, of variations of room temperature, and of a psychological stress (Stroop test). Cutaneous blood flow was recorded with laser-Doppler probes. Data are expressed as cutaneous vascular conductance (CVC) in mV/mmHg.
The results will be presented for the first time at the 25th Conference of the European Society for Microcirculation.

PCA20

SODIUM NITROPRUSSIDE (SNP) IONTOPHORESIS ON THE FINGER PAD DOES NOT INCREASE SKIN BLOOD FLOW IN HEALTHY CONTROLS AND PATIENTS WITH SCLERODERMA (SSc)

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Unlike human forearm, there is no standardized tool to assess the non endothelial-dependent skin microvascular function on the finger pad. We aimed to validate SNP-iontophoresis on the finger pad compared to the forearm in healthy controls and patients with digital microvascular dysfunction (SSc).
SNP and NaCl iontophoresis (20 seconds-100 µA, twice) were performed on the finger pad and the forearm of 6 SSc patients and 6 controls, with and without lidocaine/prilocaine cream. Cutaneous blood flow was simultaneously recorded using laser Doppler flowmetry. Data were expressed as cutaneous vascular conductance (CVC) and percentage of maximal CVC.
In both groups, SNP iontophoresis induced an increased CVC on the forearm, but not on the finger pad. We also observed an increased CVC after the iontophoresis of NaCl on the forearm but not on the finger pad. Lidocaine/prilocaine did not affect CVC after the iontophoresis of SNP on the forearm. However, it abolished the NaCl iontophoresis-induced vasodilation on the forearm.
The iontophoresis of SNP leads to a non axon reflex-dependent vasodilatation on the forearm, but not on the finger pad, both in patients with SSc and controls. As a consequence, we should consider other tools to assess the non endothelial-dependent vasodilation on finger pads.

Tuesday, August 26th
Pulmonary microcirculation
Poster Session - PPC

PPC1

MECHANISMS OF HYPOXIC PULMONARY VASOCONSTRICTION IN ISOLATED RAT INTRAPULMONARY ARTERIES

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Hypoxic pulmonary vasoconstriction (HPV) maintains the ventilation-perfusion ratio during alveolar hypoxia. We studied the mechanisms involved in intrapulmonary arteries (500–800 µm dia.) mounted in a myograph. Drugs were applied 20 min before hypoxia (95%N_2/5%CO_2; pO_2=15-20 Torr). The L-type Ca^{2+} antagonists diltiazem (10 µM; n=5) and nifedipine (3 µM; n=5), and the store-operated Ca^{2+} channel antagonist La^{3+} (100 µM; n=4) had no significant effect on HPV. HPV persisted in Ca^{2+}-free Krebs containing EGTA (200 µM; n=4; n.s.). Ryanodine (100 µM; n=4; P<0.05), the lysosomal H^+-ATPase inhibitor concanamycin A (1 µM; n=5; P<0.05) and the lysosomal degrader gly-phe-β-naphtylamide (GPN; 1 mM; n=5; P<0.05) strongly suppressed HPV, but neither the cyclic ADP-ribose antagonist 8-bromo-cADPR (30 µM; n=4) nor the AMP-activated protein kinase (AMPK) inhibitor compound C (10 µM; n=5) had a significant effect . The rho-kinase inhibitor Y-27632 (1 µM) markedly attenuated HPV (n=5; P<0.05), and hypoxia caused a biphasic increase (at 2 and 30 min) in the phosphorylation of the myosin phosphatase targeting subunit MYPT-1. These results are consistent with a model in which HPV in these arteries requires neither AMPK activation nor the influx of extracellular Ca^{2+}, but Ca^{2+}-sensitisation and intracellular Ca^{2+} release from lysosomal and ryanodine-sensitive compartments.

PPC2

HOW DOES CHRONIC HYPOXIA ACTIVATE NFAT IN PULMONARY ARTERIES?

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Chronic hypoxia (CH) leads to pulmonary hypertension. We demonstrated that NFATc3 is activated in PA from CH mice mediating CH-induced PA remodeling with increased -actin expression. Calcium-dependent activation of calcineurin is required for NFAT activation. Endothelin-1 (ET-1) is a potent activator of NFAT and is increased in CH. RhoA/Rho kinase (ROK) pathway has been implicated in NFAT activation. Our aim was to evaluate the mechanisms of CH-induced NFAT activation in PA. 9xNFAT-luciferase reporter mice were exposed to normoxia (N, 630 torr) or CH (380 torr). Prepro ET-1 mRNA was measured by real time PCR showing that 2, 7 and 21 days of CH exposure significantly increases preproET-1 in PA. In addition, animals were treated with the calcineurin/NFAT inhibitor cyclosporin A (25 mg/Kg/day); the endothelin A (ET_AR) receptors antagonist BQ123 (0.06 mg/kg/day); the L-type calcium channel blocker diltiazem (100 mg/kg/day); the ROK inhibitor HA-1077 (30 mg/kg/day) or vehicle (V). CsA and diltiazem blocked CH-induced NFAT activation. HA-1077 attenuated CH-induced NFAT activation. However, co-treatment with diltiazem did not show additive effects suggesting other pathways besides calcium and ROK may be implicated. Although CH increases ET-1, ET_AR may not be implicated in CH-induced NFAT activation since BQ123 had no effect. Alternatively, BQ123 did not effectively inhibit ET_AR.

Supported by American Lung Association and National Institute of Health (5R01HL088151-02).

PPC3

SELECTIVE LOSS OF HYPOXIC PULMONARY VASOCONSTRICTION OF MURINE INTRA-ACINAR ARTERIES IN CULTURED PRECISION-CUT LUNG SLICES

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Hypoxic pulmonary vasoconstriction (HPV) matches ventilation to perfusion by diverting blood flow to better ventilated areas of the lung. Previously, we showed that precision-cut murine lung slices (PCLS) are suitable to study HPV of intra-acinar arteries (IAA) and that mitochondrial complex II could serve as the primary oxygen sensor. Unexpectedly, in PCLS cultured at normoxia (21% O_2) and hyperoxia (95% O_2), HPV of IAA was selectively lost after 24 h, whereas the response to a thromboxane analogue (U46619) remained unaffected. The loss of HPV might be caused by altered gene expression of the mitochondrial complex II subunits (SDH-A,-B,-C,-D). PCLS and laser-microdissected IAA were subjected to qRT-PCR. Expression of each SDH subunit was significantly increased in the whole PCLS, but not in microdissected IAA. SDH-A protein expression, analyzed by Western Blotting and densitometry, was transiently increased in PCLS cultured under hyperoxia. Histochemical detection of SDH enzyme activity showed reduced activity in the airways of cultured PCLS. In the IAA, no alteration was detected. These data demonstrate that cultured PCLS can serve as a model to screen for oxygen sensor candidates. The selective loss of HPV of IAA in PCLS is not caused by altered gene expression of mitochondrial complex II subunits.

PPC4

INVOLVEMENT OF RHO-KINASE AND GLUCOSE IN HYPOXIC PULMONARY VASOCONSTRICTION IN INTRAPULMONARY ARTERIES

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Hypoxic pulmonary vasoconstriction (HPV) maintains ventilation/perfusion ratio in the pulmonary circulation. Previously we showed the dependence between HPV in intrapulmonary arteries (IPA), glucose, and Rho-kinase-mediated calcium sensitization. We examined whether changes in glucose concentration during hypoxia would also preferentially modulate vasoconstriction induced by PGF2alpha, an inducer of Rho-kinase-mediated Ca^{2+} sensitization in IPA.
Studying the effect of glucose deprivation on the response to depolarization with 80mM K or constriction with PGF2alpha (0-100 microM) using small vessel myography we observed that the response to 80mM K was reduced by ~40% of the control (60 minutes hypoxia, 0mM glucose), whilst the response to PGF2alpha was completely abolished. Inhibition of Rho-kinase with Y27632 (3microM) in normoxia reduced the response to 80mM K by ~30% in 5mM glucose, and abolished it for concentrations of PGF2alpha below 20microM. Indeed, glucose deprivation did not alter the elevation of calcium induced by PGF2alpha during hypoxia. Consistently PGF2alpha-induced phosphorylation of the myosin-binding subunit (MYPT-1) was abolished during hypoxia and glucose deprivation, as quantified by Western blot.
Conclusion: glucose deprivation during hypoxia selectively suppresses Rho-kinase-mediated calcium sensitization compared to those involving elevation of cytosolic calcium, suggesting that glycolysis is its main support. Supporters: the Wellcome Trust and Postdoctoral Grant MEC-2007-1180.

PPC5

CHRONIC HYPERCAPNIA DOWNREGULATES ARGINASE EXPRESSION AND INCREASES PULMONARY ARTERIAL SMOOTH MUSCLE RELAXATION IN THE NEWBORN RAT

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Permissive hypercapnia is a widely utilized ventilatory management strategy in newborns, applied with the goal of preventing lung injury. The mechanisms responsible for potential beneficial effects of hypercapnia on the lung are not well understood, but may relate, at least in part, to changes in pulmonary vascular function. Arginases compete for L-Arginine with endothelial nitric oxide synthase (eNOS) and their increased activity reduces eNOS-dependent nitric oxide production. Hypothesizing that hypercapnia promotes vasodilation by inhibiting arginase activity, we evaluated newborn rats chronically exposed for 14 days to either room air (control; mean $PaCO_2$ = 46 mm Hg) or 5.5 % CO_2 (CO_2 group; mean $PaCO_2$ = 70 mm Hg). Chronic CO_2 exposure significantly reduced the intrapulmonary artery arginase activity from 16±2 to 11±4 mUnit/mg of protein ($P<0.01$) and lung arginase I / actin expression ratio from 90±10 to 62±7 ($P<0.01$). Furthermore, pre-contracted (with U46619) third generation pulmonary arteries derived from CO_2-exposed animals relaxed in response to the endothelium-dependent agonist acetylcholine, significantly more than controls ($P<0.01$). We conclude that chronic hypercapnia suppresses arginase activity in the pulmonary vasculature allowing for greater relaxation potential. These results may help explain the beneficial effects of permissive hypercapnia on systemic oxygenation and lung injury observed in neonates.
Supported by grants from the Canadian Institutes for Health and Research

Tuesday, August 26th
Oxygen transport to tissue
Poster Session - POT

POT1

MATHEMATICAL MODEL OF TISSUE OXYGENATION IN SKELETAL MUSCLE OF OBESE DIABETIC AND LEAN ZDF RATS

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It has been previously reported that type II diabetes may contribute to impaired regulation of oxygen delivery. Experimentally capillary blood velocities were significantly different in 7-week old lean (ZDF-380) and obese (ZDF-370) rats (157.1 +/- 29.4 and 114.7 +/- 30.7 respectively, n = 7). The goal of the present study was to integrate in vivo experimental data into a finite difference model of convective and diffusive oxygen transport. An arteriole to venule network (30 vessel segments, in a volume of 245x562x65 micrometers) was reconstructed from video sequences of blood flow in skeletal muscle. Hemodynamic and saturation data was incorporated into the model and scaled to match conditions observed in the lean and obese groups. The model predicted a small decrease in mean tissue pO2 values in the obese vs. lean simulations (36.1 +/- 3.8 and 37.2 +/- 3.7 mmHg respectively, n = 2). In this study a 37% decrease in flow velocity did not result in an equivalent drop in tissue pO2. Further simulations examining additional networks and the impact of vessel rarefaction are necessary to more accurately determine how these changes alter tissue oxygenation in our obese diabetic rat model. Supported by NIH grant to Dan Goldman and Christopher G. Ellis

POT2

PHOSPHORESCENCE QUENCHING PO_2 MEASUREMENTS IN STATIONARY AND MOVING FLUIDS IN THE MICROCIRCULATION

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In phosphorescence quenching microscopy (PQM) frequent excitations of the same volume produce the integration of individual oxygen consumption artifacts. The error of the PO_2 measurements is determined by the radius of the excitation area R and flash rate F. The performance of PQM instruments is analyzed in order to explain reported paradoxical PO_2 results. The large R and F reduce PO_2 in stationary tissue by 36 %, creating an apparent transmural PO_2 drop previously attributed to extraordinary O_2 consumption by the vessel wall. The PO_2 underestimate caused by multiple excitations also occurs in the smallest microvessels due to their low linear blood velocity. This artifact is responsible in part for the reported longitudinal PO_2 gradients in arterioles, PO_2 rise in venules and low PO_2's in lymphatic microvessels. The use of PQM combining a small R with a low F prevents the PO_2 underestimation in flowing blood and lymph. Artifact-free PO_2 measurements in stationary tissues and fluids are achieved with a scanning beam that excludes multiple excitation of the same volume. Intravascular PO_2 measured in mesenteric arterioles was 65.0 mmHg and perivascular PO_2 was 62.2 mmHg. The insignificant PO_2 transmural PO_2 drop was 2.3 – 2.8 mmHg / 5 microns.
Support: AHA-0655449U and NIH-HL18292

POT3

OXYGEN GRADIENTS IN THE MICROCIRCULATION: INHOMOGENEOUS DISTRIBUTIONS RESULTING FROM CONVECTION AND DIFFUSION

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Oxygen delivery depends on both convective and diffusive transport. The complex spatial relationships among the various microvessels lead to correspondingly complex diffusive interactions. Here, the contributions of diffusive and convective transport oxygen saturation profiles within microvessels were examined using multispectral imaging to assess haemoglobin saturation (SO_2) in the mouse dorsal skin-fold chamber during intravital microscopy. This method is based on differences between absorption spectra of oxygenated and deoxygenated haemoglobin. A dedicated analysis software (**SOAP**) was used to obtain SO_2 data as 2D images. To isolate diffusive processes, laser micro-occlusion of venules was performed to stop flow. Arterial SO_2 values were relatively homogeneous, close to 90%, whereas venular values ranged from about 40% to 65%. In larger venules (diameter > 15 µm) flow regions with different saturation levels, showing streamlines of SO_2, could be observed. Streamlines extend over long distances, up to >1 mm. SO_2 levels on the arterial side of venules are higher than on the opposite side. In regions with low velocity, diffusive oxygen transport between arterioles and venules prevails, whereas at normal velocity convection dominates and effects of diffusion are limited. Upon flow cessation, a time-dependent increase of SO_2 due to diffusion from neighbouring arteries was observed.

POT4

WHAT IS THE CLINICAL SIGNIFICANCE OF SATURATION ($S_{mb}O_2$), THE MEAN OXYGEN CONTENT OF BLOOD?

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Mean blood saturation ($S_{mb}O_2$) and tissue oxygen index (TOI) derived by optical reflectance spectroscopy (ORS) and near infrared spectroscopy respectively evoke a concept of the measurement of oxygen delivery to tissue. This study aims to establish whether $S_{mb}O_2$ is an appropriate indicator of tissue oxygenation. Spontaneous fluctuations in $S_{mb}O_2$ observed as changes in concentration of oxyhaemoglobin [HbO_2] and deoxyhaemoglobin [Hb] were measured by ORS in the skin microcirculation of 30 healthy subjects (15 male, age 21-42 years). Wavelet analysis identified two distinctly different spontaneous falls in $S_{mb}O_2$. Type I swings induced by fluctuations in arterial blood volume resulted from the effects of respiration, endothelial, sympathetic and myogenic activity. There was no apparent change in [Hb]. In contrast, type II swings resulted from a fall in [HbO_2] accompanied by a rise in [Hb] and were only induced by endothelial and sympathetic activity. Thus the same fall in $S_{mb}O_2$ can be induced by two distinct mechanisms. A type I swing does not suggest an inadequacy in oxygen delivery whilst a type II swing may indicate a change in oxygen delivery from blood to tissue. Blood oxygen saturation cannot therefore be accepted as a definitive marker of tissue oxygenation.

POT5

MICROVASCULAR HEMODYNAMICS AND TISSUE OXYGENATION DURING EXPERIMENTAL EMBOLISM

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The pathophysiology of arterial gas embolism (AGE) involves changes in tissue blood flow and oxygenation. However, no study directly examines these parameters in the microcirculation during AGE. This study describes the initial experiments on the development of a model to characterize microcirculatory responses during experimental AGE. The femoral artery and jugular vein were cannulated in anesthetized rats. The cremaster muscle was exteriorized, placed on a heated platform. Systemic parameters were measured and noninvasive optical techniques allowed measuring microvascular diameter, red blood cell velocity, oxygen partial pressure (pO2) and micro-bubble dynamics. After baseline, controlled local application of air bubbles was performed so that some microvessels were occluded. Measurements were made before AGE and at various intervals thereafter. Most bubbles were lodged in small arterioles (≤ 30 micrometers). Intravascular pO2 during baseline averaged 80-90 mmHg for feeding arterioles and 70-80 mmHg for arteriolar side before lodged bubble. Arteriolar pO2 after lodged bubble was 20-40 mmHg. Interstitial pO2 drops were also observed. After distal bubble displacement, arteriolar pO2 was restored. The methodology allowed digital imaging of microvessels and measurements of microhemodynamics, pO2, oxygen saturation and bubble dynamics. Different therapeutics, in a variety of AGE protocols, can be tested using the model.

Tuesday, August 26th
Haemorheology and red blood cells
Poster Session - PRB

PRB1

ELEVATED RBC AGGREGATE STRENGTH IN SICKLE CELL DISEASE AS A FACTOR IN MICROVASCULAR OCCLUSION DURING CRISIS

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A hallmark of sickle cell disease (SCD) is periods of painful crisis due to microvascular occlusion. The present study compared RBC deformability and aggregation indices between healthy controls (AA, n=10) and patients with either homozygous (SS, n=17) or heterozygous (SC, n=13) SCD. All patients were in steady state and transfusion-free for at least 90 days. RBC deformability (elongation index, EI) was measured at 3 and 30 Pa using a LORCA system. The LORCA was also used to obtain three RBC aggregation indices: 1) aggregation index (AI); 2) aggregation half time (t½); 3) minimal shear stress to prevent aggregation (□). Measurements confirmed the presence of rigid erythrocytes in SS and SC patients, with the lowest EI values for SS. Average values for □ were significantly elevated in SS and SC, whereas there were no significant differences of AI, t½ and fibrinogen level between the groups. A notable observation was the lack of association between higher □ and higher plasma fibrinogen levels in SCD. We suggest that specific RBC membrane factors increase aggregate strength, thereby requiring higher shear stress for dispersion: abnormalities of both RBC deformability and aggregation may thus contribute to the disturbed microcirculatory flow observed in SCD.

PRB2

REDUCED MICROCIRCULATORY BLOOD FLOW IN PATIENTS UNDERGOING MAJOR AORTIC SURGERY IS ASSOCIATED WITH HEMOLYSIS

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Hemolysis is recognised to potentially decrease nitric oxide (NO) bioavailability leading to compromised microcirculatory blood flow. The relevance of hemolysis to the microcirculation in patients undergoing major aortic surgery with extracorporeal circulation and massive transfusion remains unknown. Aims of the study were to determine hemolysis in these patients by assessment of intravascular hemolytic products arginase-1 and Free hemoglobin (Free Hb), and to evaluate the putative physiologic effects of these products on NO metabolism and microcirculatory blood flow, represented by forearm blood flow (FBF) measurements. Elevated plasma arginase-1 levels in these patients did not affect plasma concentration arginine, the substrate for NO-synthesis, therefore no systemic effect on microcirculatory blood flow caused by the release of this enzyme was expected. Interestingly, impaired FBF response to an exogenous NO-donor correlated significantly with increased plasma Free Hb levels (r -0.89; P = 0.0046), whereas endothelial reactivity in all patients was normal. These findings suggest that release of Free Hb leads to quenching of NO and disturbances in microcirculatory blood flow, which might additionally contribute to organ injury in patients undergoing major aortic surgery. This study supports therapies counteracting the deleterious effects of Free Hb or enhancing NO bioavailability in patients undergoing major aortic surgery.

PRB3

DECREASED ERYTHROCYTE AGGREGATION IN MEN WITH ISCHEMIC HEART DISEASE AND NORMAL ERECTIONS

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The role of erythrocyte aggregation in the pathophysiology of erectile dysfunction (ED) in men with ischemic heart disease (IHD) has never been studied. In this study, a total of 119 men underwent coronary angiography and filled the Sexual Health Inventory for Males questionnaire (SHIM). Excluded were men with acute coronary syndromes. Erythrocyte aggregation was evaluated by filming slides of blood smear and calculating the erythrocyte percentage (EP), i.e. the field that was covered by

erythrocytes. SHIM scores 21 or less represented ED. The cohort included 62 men, mean ages 61.0±11.9 years. Mean SHIM scores were 15.5±8.1 and the mean EP was 70.9±14.7%. EP was associated with SHIM scores (r=0.4; p=0.001). After dividing the cohort into four groups: men with no ED and no IHD (n=6), men with ED but no IHD (n=9), men with both IHD and ED (n=40), and men with IHD but no ED (n=7), EP was significantly higher in the last group compared with the other groups (69.7±8.7%, 68.6±16.6%, 69.0±14.6%, and 86.4±7.1%, respectively; p=0.02). We conclude that men with IHD and normal erections have decreased erythrocytes aggregation. This finding may be the first step in establishing therapeutic intervention in order to prevent ED in men with IHD.

PRB4

THE IMPACT OF IN VITRO AGING ON ERYTHROCYTE AGGREGATION

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Hemorheological measurements should be performed quickly as storage of samples can alter the results. We investigated the effect of in vitro aging on erythrocyte aggregation. Erythrocyte aggregation of 21 samples was measured with LORCA and Myrenne devices after various in vitro aging: controls were measured immediately. Some sample fractions were stored on ambient temperate for 2, 4 and 6 hours, either standing still or being rolled continuously. Other fractions were stored in refrigerator for 1, 2 and 7 days. Myrenne aggregation parameters decreased significantly already after 2 hours (M: 13.6 +/- 2.1 vs. 12.3 +/- 2.5; M1: 22.7 +/- 2.9 vs. 21.6 +/- 3.2; p<0.001), and decreased even further afterwards. Averages of LORCA aggregation indices (AI) did not change significantly in any fractions, however, their standard deviation showed increasing tendency which became significant in the 7-day refrigerated fraction. Correlation between LORCA AI and Myrenne M1 parameters was significant in controls (r=0.591, p<0.01), but this declined and vanished afterwards. AI and M parameters showed no correlation at all. In vitro aging quickly and strongly influences erythrocyte aggregation measured with Myrenne. LORCA is much less affected, though prolonged storage may decrease the accuracy of measurements. The two methods cannot substitute each other.

PRB5

PATIENTS' AGE AND THEIR DISEASE DURATION DEPENDING ON RHEOLOGICAL PARAMETERS DURING ARTERIAL HYPERTENSION

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In our country, 18% of adults have hypertension disease, which means that the second time their ABP raises it reaches 160/95 mm Hg and higher. We learned patients with arterial hypertension (without RAS) (n=34, mean age – 8.2+/-8.7, mean disease duration – 12.4+/-4.7). We were interested in rheological parameters so in every patient's blood sample we investigated the RBC aggregability using the "Georgian Technique" (G.I. Mchedlishvili at al, Biorh. 30:153-161. 1993) and blood plasma viscosity by using Lowry Method (OH Lowry et al. Biol Chem 193:265., 1951) The RBC aggregability is 55.6+/-1.9 and blood plasma viscosity is 1.35+/-0.011) in this group. We measured the RBC aggregability and blood plasma viscosity in blood samples of healthy subjects too (n=25, control group's age - 52.4+/-8.2, RBC aggregability index - 26.8+/-6.7 and blood plasma viscosity – 1.2+/-0.01). The data showed that the RBC aggregability and blood plasma viscosity in the group of patients with arterial hypertension was significantly higher in comparing with the control group (p<0.001). But we determined that the RBC aggregability and blood plasma viscosity increases are not connected with patients' ages but depend on the straight-line relationship with the disease duration. Our data lets us know that the longer a subject has a hypertension disease the clearer the rheological disorders can be seen, so every patient, no matter the age, should contact a doctor first time his/her ABP raises, so the doctor could make corrections in his/her treatment for rheological disorders.

PRB6

WHOLE BLOOD HEMORHEOLOGICAL CHANGES IN COLD AND WARM ISCHEMIA REPERFUSION INJURY OF THE HIND LIMB

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Rheology of the blood changes in different physiological and pathological conditions and it influences blood supply of the effected tissues. In the present studies we examined changes of hemorheological alterations caused by ischemia reperfusion injury (IRI). 24 anesthetized dogs had an acute and complete hind limb ischemia for 3 hours followed by early (4 hours) and late (5 days) reperfusions. In one group ischemia was carried out in cold (5 °C) and in another in normal (22 °C) temperature conditions. Parallel control groups did not have ischemia. Venous blood samples were collected at early and late reperfusion periods for rheological analyses: whole blood and plasma viscosity (WBV, PV), relative cell transit time (RCTT) of erythrocytes as a measure of red cell deformability, fibrinogen concentration, and hematology. RCTT increased in IRI peaking at 2, 3 days of reperfusion, cold ischemia induced higher elevation. WBV was near constant and it increased in cold IRI at the end of reperfusion. Fibrinogen concentration also increased in late reperfusion in both cold and warm IRI groups. No major differences were found in the hematological parameters among the different groups. It is concluded that, although, cooling may increase ischemic tolerance, cold IRI unfavorably affects the microcirculation of skeletal muscle. (OTKA T-32571).

PRB7

HEMORHEOLOGIC AND THROMBOSIS PREVENTIVE POTENTIAL OF CHICORY COFFEE CONSUMPTION: A CLINICAL STUDY

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Protective effects of plant polyphenol intake, (e.g. berries or wine), on cardiovascular morbidity are inevitable. The caffeine-free chicory coffee contains several of these phenolics, e.g. caffeic acid, that inhibits platelet aggregation in vitro. We have shown previously that caffeic acid inhibits the tautomerase enzymatic activity of the proinflammatory cytokine, macrophage migration inhibitor factor (MIF). In the study reported here 27 healthy volunteers were recruited to consume 300 ml chicory coffee every day for one week. ADP induced platelet aggregation increased compaired to baseline both, at 2 hours after the first cup and after one week coffee consumption. The collagen induced platelet aggregation was decreased from baseline at 2 hours, however, the epinephrine induced aggregation was increased. Both, whole blood and plasma viscosity showed significant decrease after one week of chicory coffee consumption, moreover, significant improvements were seen in red blood cell deformability. No changes in hematocrit, fibrinogen level or red blood cell counts were detected. A significant decrease was observed in MIF levels after one week compared to either baseline or 2 hour levels. Our study gives an encouraging starting-point to further delineate the antithrombotic and antiinflammatory effects of phenolic compounds of chicory coffee.

Tuesday, August 26th
Shock
Poster Session - PSH

PSH1

PRESERVATION OF ORGAN FUNCTION AND REDUCED MORTALITY BY INTRAINTESTINAL PANCREATIC DIGESTIVE ENZYME INHIBITION IN SEPTIC SHOCK

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Despite advances in the understanding of the pathophysiology and treatment of septic shock, knowledge of the complex mechanisms that are involved in the inflammatory response is lacking. The gastrointestinal tract plays a central role during septic shock. The intestinal wall generates inflammatory mediators which may be involved in the prolonged inflammation during sepsis. The pancreatic proteases generate and sustain systemic cell activation with consequent organ injury. But in septic shock the impact of pancreatic derived inflammatory mediators on preservation of organ function and mortality is unexplored. To determine the role of inflammatory mediators generated pancreatic enzymes in septic shock, examination of digestive enzyme blockade in the lumen of the intestine was investigated in adult rats. One hour after endotoxin administration, the small intestine was subjected to intraluminal loading with and without an inhibitor of pancreatic proteases and lipases. Without blockade of digestive enzymes, more than 90% of the animals died within the first 24 hours, while with blockade more than 70% survived for a period of 2 weeks at which time animals had returned to normal weight gain. These results show a dramatic reduction of mortality rates in endotoxin induced multi-organ failure by a procedure with possible therapeutic utility. (Supported by a gift from Leading Ventures, San Diego, California)

PSH2

CHANGE OF LYMPH MICROCIRCULATION IN RAT WITH ANAPHYLACTIC SHOCK OF VARIOUS OVERCOME

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Dept. of Pathophysiology, Hebei North Univ. China.

The anaphylactic shock model was established by intensified sensitization of sheep serum and adjuvant. Owing to the species characteristic of low allergy in rats, 45.5% rats had irreversible shock, 54.5% rats had reversible shock. The microcirculatory change showed that blood pressure reduction, but the contractility of mesenteric microlymphatics in irreversible shock rats was significantly decreased and even disappeared at the early stage of anaphylactic shock, while in the reversible shock group, the contractility was only mildly and transiently decreased, and was retrieved before blood pressure rebounded, the mesenteric lymphatic flow and lymphatic protein output was also higher than the irreversible shock group. The ultrastructure of initial lymphatic endothelial cell in the irreversible shock group was severely damaged, while in the reversible shock group, this damage was slighter. It indicated that the function of lymph microcirculation plays important role in shock development and its reverse. A perfect function of lymph microcirculation contributes to shock reversion, and lymph microcirculatory dysfunction aggravates shock or even leads to death. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

PSH3

CHANGES OF CONTRACTILITY OF MESENTERIC MICROLYMPHATICS IN HEMORRHAGIC SHOCK RATS AND ITS MODULATION

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The hemorrhagic shock model was established, the caliber of mesenteric microlymphatics (ML) was observed by alive-body lymph microcirculation technology, to calculate the lymphatic contractile activity index, overall lymphatic constractile activity index, lymphatics dynamics index and contraction frequency. The change of ML responses to norepinephrine (NE) was observed by that different concentration from low to high NE was dropped locally on the mesentery, and effect of denervation using splanchnicectomy on contractility of ML in shock phase. The results showed that the contractility and responses of ML were normal in shock forepart. Get along with shock, diameter, spontaneous contractility and responses of ML were decreased gradually. After denervation, the ML spontaneous contraction frequency, contractility decreased significantly. During refusion of withdrawn blood and infusion of normal saline periods, the ML spontaneous contractility in the intact group was significantly higher than pre-shock, but it remained at a level in denervated group, lower than intact group. During post-infusion period, the blood pressure was still much lower in denervated group than intact group. The results demonstrated that the contractility and responses of ML were reducing in severe shock phase and its modulation by sympathetic-adrenal medulla axis. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

PSH4

MECHANISM OF MESENTERIC LYMPH REPERFUSION ON ORGANS INJURY IN SUPERIOR MESENTERIC ARTERY OCCLUSION SHOCK RATS

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Dept. of Pathophysiology, Hebei North Univ., China.

Intestinal lymphatic pathway played an important role in the pathogenesis of superior mesenteric artery occlusion (SMAO) shock. The SMAO shock model was established and observed the mechanism of mesenteric lymphatic reperfusion (MLR) facilitating organs injury of SMAO shock. The results showed that the MDA, NO contents of lung, kindey, myocardium homogenate, the NOS activity of liver homogenate, the MPO activity of kidney, liver homogenate in SMAO and MLR+SMAO group were increased significantly than Sham and MLR group, and the MDA, NO, MPO contents of lung, kidney, liver, myocardium homogenate, the NOS activity of lung, kidney, myocardium homogenate in MLR+SMAO group were higher obviously than SMAO group. The SOD and ATPase activity of muliple organs homogenate in SMAO and MLR+SMAO group were lower than Sham and MLR group, and that of MLR+SMAO group was decreased than SMAO group, obviously. The results demonstrated the mechanism of the MLR could aggravate the major organs injury of SMAO shock might be that the MLR enhance the multiple organ free radical injury, NO synthesis and release, PMN detention and decrease the activity of Cell Membrane ATPase. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

Tuesday, August 26th
Lymphatics
Poster Session - PLY

PLY1
EXPLORING THE ACTIVE REGULATION OF LIPID TRANSPORT BY LYMPHATICS WITH A NOVEL IN VITRO MODEL
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Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland.

The role of lymphatics in lipid uptake and transport in the gut is well appreciated, but the biological regulation of lipid transport across lymphatic endothelium is poorly understood. We present an in vitro model of the intestinal microenvironment using Caco-2-based enterocytes and lymphatic endothelial cells (LECs) separated by a thin collagen barrier. We characterize this model for quantifying chylomicron transport using a fluorescent fatty acid probe, bodipy, from the intestinal to lymphatic compartments and explore relative contributions of each cell type as well as their cross-talk in mediating lipid, protein, and sugar transport. We show that the synthesis and secretion of chylomicrons is polarized for preferential transport from intestine to lymphatic direction. Furthermore, chylomicron-containing enterocyte secretions increase transepithelial LEC transport of albumin but not small dextrans. LECs, in turn, affect chylomicron synthesis by enterocytes. Characterizing the active roles that lymphatics play in lipid uptake in the gut and reverse cholesterol transport in the interstitium will be crucial for developing new treatments for the complications that arise from severe obesity and lymphedema, as well as for developing new strategies for the targeting of intestinal lymphatics with novel oral drug delivery systems.

PLY2
LONG-TERM CULTURE OF LYMPHATIC VESSELS AND DEVELOPMENT OF TRANSFECTION TECHNIQUES TO TARGET GENES INVOLVED INTO REGULATION OF LYMPHATIC CONTRACTILITY
AA Gashev [1], P Dougherty [1], OYu Gasheva [1], ZV Nepiushchikh [1], W Wang [1], KA Kelly [1], S Cai [2], M Periasamy [3], GJ Babu [3], CJ Meininger [1], MJ Davis [4], M Muthuchamy [1], DC Zawieja [1]
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The power and specificity of gene transfection has never been tested on lymphatic vessels (LV). We have optimized the organ culture technique to maintain the viability of isolated rat LV for 7-9 days that would be sufficiently long to overexpress or knock down genes of interest. We used adenovirus-expressing GFP to test the transfection efficiency - ~80-90% GFP expression in lymphatic muscle cells with typical LV contractility patterns. We transfected several genes involved in the regulation of lymphatic contractility. After 72 hours of overexpression of SERCA2a in LV muscle cells the functional tests demonstrated diminished lymphatic tone (predicted lowered cytoplasmic Ca++) and ~50% higher lymphatic phasic amplitude (predicted enhanced systolic Ca++ release). We specifically knocked down of 80-95% MLCK in LV - both tone and phasic contractions were greatly diminished. Further refinement of the organ culture conditions allowed us to perform long-term monitoring (up to 8 days) of isolated LV under controlled pressure condition. This experimental model allowed us to establish controlled, high-pressure conditions (10 cm H_2O) for 4-5 days with first reversible signs of pump failure after 2-3 days; model can be used as an accessible and reasonable simulation of the lymphatic pump alterations during lymphedema.
Support: NIH RO1 HL070308 and HL080526.
Keywords: lymphatic vessels, lymphatic contractility, transfection, SERCA2, MLCK, organ culture, experimental lymphedema model.

PLY3
AGING AND LYMPHATIC CONTRATILITY
OYu. Gasheva [1], E Rahbar [2], ZV Nepiyushchikh [1], M Muthuchamy [1], AA Gashev [1]
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All functions of lymphatic system require the lymph flow. This flow can not exist without the driving force generated by contractions of lymphatic vessels (LV). However, the mechanisms regulating the lymphatic contractility remain greatly under discovered and particularly the mechanisms of age-related alterations in lymphatic pumping remain unclear. Using confocal imaging technique, we observed the profound reduction of muscle cells in aged rat LV. In vivo flow measurements using fast video microscopy demonstrated greatly decreased basal lymph flow; maximal lymphocyte velocities in aged mesenteric LV are 4-6 times lower than in the adult LV, contraction amplitude diminished by 50-60%; while the contractions are irregular with long periods of inactivity. The profound inhibition of the contractile activity has been observed also in isolated aged rat thoracic duct (TD) and mesenteric LV. The basic flow/eNOS-dependent regulation is abolished in aged TD, protein message is greatly depleted. At the same time in aged TD the substantial iNOS activation, confirmed by Western blotting and immunohistochemistry, occurs; its functional importance confirmed by pressure/flow tests. We concluded that depletion of

the contractile reserves in LV in elderly diminishes their ability to provide the adequate transport of lymph during the periods of the increased volumetric loads. Support: TAMU CERH grant 5 P30 ES09106-08, NIH RO1 HL070308 and HL080526.

PLY4

MICROCIRCULATORY CONSEQUENCES OF IMPAIRED LYMPHATIC DRAINAGE IN A MOUSE MODEL OF PRIMARY LYMPHEDEMA

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Lymphedema is a chronic condition characterized by swelling of the affected limb due to insufficient lymph drainage accompanied by fibrosis and susceptibility to infections. The pathophysiology of this condition is incompletely understood. We investigated transcapillary fluid balance parameters in a transgenic mouse model of primary lymphedema, the K14-VEGFR-3 Ig mouse, which due to an inhibition of VEGF-C/VEGF-D-signalling in keratinocytes, lacks dermal lymphatics. In adult young mice (3-4 months), the interstitial fluid pressure (P_{if}), measured with micropipettes, and tissue fluid volumes were significantly increased in the areas of visible swelling (the fore and hind paw) only, whereas the interstitial colloid osmotic pressure (COP_{if}) was increased compared to wild type (WT) mice in all skin areas examined (fore paw, hind paw, thigh and back skin). In more longstanding lymphedema (age 12-15 months), P_{if} was similar to that of young adult lymphedema mice whereas there was a concomitant increase in COP_{if}. Proinflammatory cytokines (IL-2, IL-6, IL-12) in subcutaneous interstitial fluid in the paw were lower in transgenic than in WT mice. These data indicate a reduced immunological defence ability or response in skin in this model of primary lymphedema that may have implications for our understanding of the pathophysiology of this condition.

PLY5

ADHESION MOLECULES INVOLVED IN INTRAVASATION OF TUMOR AND DENDRITIC CELLS INTO LYMPHATICS.

OD Miteva, MA Swartz

Inst. of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

Lymphatic vessels are the primary route for dendritic cell (DC) trafficking and breast cancer metastasis, but active mechanisms that govern interactions between lymphatic endothelial cells (LECs) and migratory cells are not well understood. Here we address the questions of whether tumor cells and DCs use similar molecular mechanism to adhere to and intravasate into the lymphatic vasculature, and what those mechanisms are. We used monocyte-derived human DCs, metastatic human breast cancer cell line MDA-MB-435S, and nontumorigenic cell line MCF10A. We found that while DCs and the MCF10A stimulate ICAM-1 and VCAM-1 expression on LECs, MDA-MB-435S did not; these molecules are required for focal and firm adhesion in blood endothelium. E-selectin was not expressed on LECs, even in the presence of tumor cells, but was upregulated in presence of DCs. Furthermore, we show how these molecules are regulated by small levels of interstitial flow, and how their activity depends on flow conditions: those experienced by normal drainage function and also elevated flow- inflammatory conditions. We conclude that ICAM-1,VCAM-1 and E-selectin may play an important role in the transmigration of DCs but are differentially regulated in transmigrating tumor cells, and that flow is an important signal to lymphatics in regulating these molecules.

PLY6

EFFECTS OF ETHANOL EXTRACT FROM *MRRUBIUM INCISUM* ON ACUTE MICROCIRCULATORY DISTURBANCE IN RATS

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Dept. of Pathophysiology, Hebei North Univ., China.

The acute microcirculatory disturbance model was established by intravenously administrating Dextran 500 (10ml/kg.bw). 6 minutes later, ethanol extract from *Mrrubium Incisum* (EEMI) was infused in *Mrrubium Incisum* group (1g/ml, 6g/kg), and EEMI was replaced by normal saline in model group. The mesenteric blood and lymph microcirculation were observed by intravital microscope with TV recorder. Compared with before experment, the diameter of mesenteric microvessels was contracted, the micro-blood flow speed was slowed, the RBC aggregated, and the value was higher in model group; at the same time, the spontaneous contraction frequency, lymphatic constractile activity (Index I), total lymphatic constractile activity (Index II) and the lymphatics dynamic (LD-Index) of mesenteric microlymphatics (ML) were reduced observably. After EEMI injection, the diameter of mesenteric microvessels was expanded, the micro-blood flow speed was quickly and the value was lower in *Mrrubium Incisum* group than model group, and the spontaneous contraction frequency, Index I, Index II and LD-Index of ML were heightening observably. The results demonstrated EEMI could lighten the acute blood and lymphatic microcirculatory disturbance subjected to Dextran 500. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

Keywords: *Mrrubium Incisum* Benth; acute microcirculatory disturbance; dextran 500; blood microcirculation; lymph microcirculation

PLY7

DIFFERENTIAL TISSUE ADAPTATION IN TWO MOUSE MODELS OF PRIMARY CONGENITAL LYMPHEDEMA WITH DEFECTS IN VEGFR-3 SIGNALING

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Primary lymphedema is a congenital pathology of dysfunctional lymphatic drainage characterized by swelling, dermal thickening, and tissue fluid and lipid accumulation. Two mouse models of lymphedema, the Chy mouse and the K14-VEGFR-3-Ig mouse, target vascular endothelial growth factor receptor (VEGFR)-3 signaling, and exhibit dermal lymphedema symptomatically similar to the human condition. Chy mice possess a genetic mutation in VEGFR-3, while K14-VEGFR-3-Ig mice express soluble VEGFR-3; both models subsequently lack dermal lymphatic capillaries. We sought to quantitatively determine the functional interstitial transport implications resulting from these mutations. We found that despite their similarities in increased skin hydration and elevated interstitial fluid pressure, the dermal tissue adaptations were significantly different. Chy mouse skin possessed much higher levels of collagen and fat, which normalized hydraulic conductivity, while the fat and collagen content in K14-VEGFR-3-Ig skin was relatively normal, leading to a greatly increased conductivity. Thus, we hypothesize that Chy mice have adapted to lack of initial lymphatics by decreasing interstitial transport, while K14-VEGFR3-Ig mice have adapted to increase or facilitate interstitial flow over longer distances. These responses to reduced lymphatic drainage (via missing initial lymphatics) suggest that tissue remodeling in lymphedema is not simply a consequence, but an adaption to limit tissue swelling.

PLY8

LYMPHOEDEMA AFTER BREAST CANCER TREATMENT IS ASSOCIATED WITH A PRE-EXISTING HIGH-FILTRATION STATE

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Subcutis and muscle lymph flow declines in established breast cancer-related lymphoedema (BCRL). We investigated lymph flow in the window between surgery and BCRL onset in 36 non-oedematous patients 7m after axillary surgery. ^{99m}Tc-human IgG (^{99m}Tc-HIG) was injected into the subcutis of each forearm. Removal rate constant k, an index of lymph flow/distribution volume, was measured by lymphoscintigraphy. Intra-muscular k was measured 7 days later. Measurements were repeated at 30m post-surgery, when 7/36 women had BCRL. In women without BCRL k_{muscle} was 2-3x $k_{subcutis}$, i.e. muscle contributes the greater lymph load. At 7m there were no between-arm differences in k, even in women destined for BCRL, hence no evidence of pre-BCRL lymphatic failure. Unexpectedly, at 7m k_{muscle} and $k_{subcutis}$ were 22-50% higher in both arms of the pre-BCRL group than in the women who did not develop BCRL (P<0.01, 2-way ANOVA). Subsequent to BCRL onset, $k_{subcutis}$ fell in both arms; k_{muscle} did not. In women spared BCRL, $k_{subcutis}$ and k_{muscle} increased from 7m to 30m. The results indicate that women destined to develop BCRL have elevated lymph formation rates, and presumably high capillary filtration rates. This is the first evidence for a subgroup constitutively predisposed to secondary lymphoedema.

Wednesday, August 27th
Diabetes mellitus, obesity
Poster Session – PDO

PDO1

EFFECTS OF CURCUMIN ON GINGIVAL MICROCIRCULATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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This study was conducted to investigate the effect of daily feeding of curcumin on gingival microcirculation in diabetic rats. Wistar Furth rats were divided into three groups: CON (single injection of citrate buffer), DM (streptozotocin, 50 mg/kg.BW, i.v.), DM+cur (daily oral treatment of 300 mg/kg.BW curcumin). On 8th week after streptozotocin injection, laser Doppler flowmetry was used for gingival blood flow (GBF) measurement. Using intravital fluorescence videomicroscopy, leukocyte adhesion in the venule was examined for each group. Blood glucose (BG), glycosylated hemoglobin (HbA1C), lipid profiles were measured in blood samples collected at the end of each experiment. The contents of liver malondialdehyde (MDA) were also quantified for each group. Gingival blood flow in DM rats decreased significantly compared to the corresponding level of CON rats. Treatment with curcumin could restore the decrease of GBF. The leukocyte adhesion seem to be increased in DM and DM+cur rats compared to CON rats. However, no significant differences were observed. Feeding curcumin (DM+cur) could decrease BG, HbA1C , dyslipidemia and MDA significantly, compared to DM rats. In conclusion, curcumin might have the effect in protecting the abnormality in gingival microcirculation of diabetic rats through its antioxidant with hypoglycemic and hypolipidemic actions.
This work was supported by the Dental Faculty Research Fund, Chulalongkorn University. Bangkok, Thailand.

PDO2

CORONARY MICROVASCULAR ADAPTATION IN OBESITY

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In metabolic syndrome presence of obesity and hypertension have an impact on the function of vascular system, but their effects on the function of coronary microvessels are poorly understood. Thus, we investigated the vasomotor function of coronary arterioles isolated from the heart of patients who underwent cardiac surgery. We found that endothelium-dependent vasodilator, bradykinin (BK) and the NO-donor, sodium nitroprusside (SNP) elicited substantial dilation in isolated coronary arterioles. There were no significant differences between BK-induced and SNP-evoked dilations of coronary arterioles isolated from normotensive and hypertensive individuals. However, when coronary dilations were segregated into lean (BMI<25) and obese (BMI>30) patients a marked difference could be revealed. BK-induced dilations were significantly reduced in obese-normotensive patients, when compared to lean-normotensive (P=0.013), and also when compared to obese-hypertensive patients (p=0.025). Moreover, we found that the SNP-induced dilations were reduced in lean-hypertensive individuals, when compared to lean-normotensive (P=0.017) and also when compared to obese-hypertensive patients (P=0.021). Our observations revealed that in metabolic syndrome presence of obesity and hypertension have divergent effects on the vasomotor function of coronary microvessels. Our data suggest that presence of obesity may activate adaptive vascular mechanisms, which could play a role in the maintenance of tissue perfusion in obesity.
Support: Hungarian National Scientific Research Fund (OTKA) F-048837

PDO3

ACTIVATION OF HEXOSAMINE PATHWAY IMPAIRS NITRIC OXIDE-DEPENDENT ARTERIOLAR DILATIONS

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We hypothesized that under high glucose concentrations, activation of the hexosamine pathway leads to glucosamine formation, which interferes with nitric oxide (NO)-dependent arteriolar dilation. To test this postulation, skeletal muscle arterioles (diameter: 160 m) isolated from healthy, male Wistar rats were exposed to normal glucose (NG, 5.5 mM) or high glucose (HG, 30 mM for 2 hours) concentrations and agonist-induced diameter changes were investigated. In arterioles exposed to HG concentration, dilations to histamine were markedly reduced compared to those of under NG condition. Inhibition of NO synthesis with L-NAME reduced histamine-induced dilations in NG arterioles, but it had no effect on microvessels exposed to HG. Dilations to the NO donor, sodium nitroprusside were similar in the two groups of vessels. In the presence of the inhibitor of hexosamine pathway, azaserine histamine-induced dilations were significantly augmented in arterioles exposed to HG concentrations. Moreover, under NG condition, exposure of vessels to glucosamine (5 mM, for 2 hours) resulted in reduced histamine-induced arteriolar dilations. These findings indicate that high glucose concentration may lead to glucoseamine formation, which impairs histamine-induced, NO-mediated arteriolar dilations. We propose that interfering with the hexosamine pathway may prevent microvascular complications in diabetes mellitus.

PDO4

ATTENUATION OF MURINE AORTIC VESSEL RESPONSES IN THE PRESENCE OF PROTEINS MODIFIED BY ADVANCED GLYCATION END-PRODUCTS.

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Hyperglycaemia is associated with impaired endothelial cell function and altered vessel contractility. This may be due to non-enzymatic glycation of proteins by glucose and its metabolite methylglyoxal (MG), leading to the accumulation of advanced glycation end-products (AGEs). We investigated the influence of AGE-modified proteins, generated from MG, on (a) the proliferative and migratory responses of bovine endothelial cells and (b) on murine aortic vessel function, using isolated aortic rings, from male Wister rats. The effects of Phenylephrine (PE, 10-7M), Potassium chloride (60 mM) and Acetylcholine (Ach, 10-6M) were assessed 30 minutes after incubation in AGE-modified BSA (AGE-BSA), MG alone, or BSA alone. Addition of BSA-AGE (10-500 microgram/ml) inhibited endothelial cell proliferation and migration in a dose dependent manner. All vessels constricted to high potassium solution. There was a significant reduction in PE induced contraction when vessels were incubated in the presence of AGE-BSA (3.08+/-0.22 and 2.17+/-0.68, $p<0.05$). The percent relaxation to Ach was also attenuated in the presence of AGE-BSA (37.40+/-0.60% and 26.04+/-1.13%, *p=ns*), but not thirty minutes after washout. Our study suggests an important role for AGEs in the modulation of vascular function and may be an important target for therapeutic intervention in the treatment of diabetic patients.

PDO5

HIGH GLUCOSE DIFFERENTIALLY REGULATES COUP-TFII IN INSULIN-PRODUCING CELLS AND VENOUS ENDOTHELIAL CELLS

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COUP-TFII regulates transcriptional activity of the rat insulin promoter. A heterozygous deletion in mouse islet ß-cells leads to impaired glucose sensitivity and abnormal insulin secretion. Furthermore COUP-TFII is specifically expressed in venous endothelium and has an influence on arteriovenous differentiation. To follow up the link between COUP-TFII and glucose homeostasis, we stimulated insulin-producing cells (INS-1) with high glucose medium (25mM). We found a significant COUP-TFII protein decrease after 30 and 60min whereas nuclear localisation was not changed. To analyse the potential link between glucose homeostasis and the function of COUP-TFII as venous marker gene, we cultured venous endothelial cells (HUVEC) in high glucose medium and stimulated them with 5nM insulin. First we verified expression of glucose transporters GLUT1, 3, 6 and 10 and insulin receptor. After stimulation for up to 60min we found a significant increase of COUP-TFII expression with or without insulin. In contrast, long term cultivation in high glucose medium with or without insulin stimulation resulted in a decrease of COUP-TFII protein and mRNA after 48h. E-selectin and insulin receptor expression were strong upregulated after long term stimulation. In conclusion, our data suggest that the venous marker COUP-TFII is regulated via glucose in venous endothelial cells.

PDO6

EP1 PROSTAGLANDIN E2 RECEPTOR ACTIVATION LEADS TO ENHANCED ARTERIOLAR TONE AND BLOOD PRESSURE IN MICE WITH TYPE 2 DIABETES MELLITUS

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We tested the hypothesis that PGE2 via activating type 1 PGE2 (EP1) receptors increases arteriolar tone and enhances blood pressure in mice with type 2 diabetes (db/db mice). We found that db/db mice exhibited elevated blood pressure and enhanced myogenic tone of isolated, pressurized gracilis muscle arterioles, when compared to controls. In db/db mice the enhanced arteriolar tone was reduced by the selective COX-2 inhibitor, NS-398 (10μM) or by the EP1 receptor antagonist, AH-6809 (10μM) to control level. In db/db mice plasma level of PGE2 metabolites were markedly elevated. In gracilis arterioles application of PGE2 (10pM-100nM) or the selective agonist of EP1 receptor, 17-phenyl-trinor-PGE2 (10pM-100nM) elicited constrictions that were significantly enhanced in db/db than in control mice. In the aorta of db/db mice an increased EP1 receptor protein expression was detected by Western blot. Moreover, oral administration of EP1 receptor antagonist, AH-6809 (10mg/kg/day) significantly reduced systolic blood pressure in db/db mice, but did not affect blood pressure of control animals. Thus, we propose that an increased COX-2 expression in resistance vessels leads to an enhanced production of PGE2, which via activating the upregulated EP1 receptors increase arteriolar tone and may elevate blood pressure of mice with type 2 diabetes.

PDO7

OSMOTIC STRESS AND DIABETIC STATE INDUCES MICROVASCULAR PRECONDITIONING.

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The committed step for microvascular preconditioning is stimulation of the mitochondrial Katp channels downstream, thus linking this response to pro-inflammatory states. We hypothesized that the pro-inflammatory state associated with murine diabetes (leptin deficient) would include a preconditioned microvasculature. The cremaster muscle of age-matched anesthetized (50mg/kg) mice (C57BL/6, n= 60; db/db, n= 9; ob/ob, n= 10) was prepared for intravital microscopy. Diminished dilation to adenosine is a hallmark signs for preconditioning in our lab. Arteriolar dilation to topical 10-4M adenosine (ADO) was decreased in db/db and to a lesser extent in ob/ob mice vs. C57BL/6. Blood glucose was significantly elevated for the db/db (390±111mg/dL) and ob/ob (238±99) vs. C57BL/6 (154±46). To test whether high interstitial glucose alone could induce preconditioning, 15mM (270mg/dL) glucose or mannitol (osmolar control) was added to the tissue bath of C57BL/6. Arteriolar dilation to topical ADO was unchanged after 2 hours exposure to glucose, but reverted to constriction with mannitol. Lastly, we tested for dilation to micropipette 10-4M L-arg, also a hallmark sign for preconditioning. L-arg induced slight constriction in timed controls, but a significant dilation with either mannitol or glucose exposure. Thus, preconditioning is induced by osmotic stress, not glucose per se. (AHA 0655908T, NIH DK68401)

PDO8

MICROVASCULAR REACTIVITY IN ACUTE HYPERGLYCEMIA IN TYPE 1 DIABETES

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High glycemic variability and high glycemic excursions are considered a risk factor for macrovascular complications in diabetes. The aim of the study was to evaluate skin microvascular reactivity (MVR) in Type 1 diabetic patients during isoglycemic and hyperglycemic hyperinsulinemic clamp to test the effect of acute hyperglycemia. Lipid parameters, fibrinogen and cell adhesion molecules (CAM) were evaluated, too.
20 patients (age 51±7 years, BMI 25±3 kg.m-2, diabetes duration 24±7 years, HbA1C 6.3±1.0%) were studied initially during isoglycemic (2 hours) and consequent hyperinsulinemic (2 hours) clamp and after two weeks during appropriate intravenous volume load as control examination to exclude non-specific factors, especially hemodilution. Skin MVR was measured by laser Doppler flowmetry continually during clamp and after post-occlusive (PORH) and thermal hyperemia (TH) at the ends of isoglycemic and hyperglycemic phase.
Venous blood glucose was 6.5±1.9 mmol.l-1 in isoglycemic and 12.0±1.6 mmol.l-1 in hyperglycemic phase of the clamp. Maximal perfusion in PORH increased in hyperglycemic phase compared to basal value (47±16 vs 40±16 PU, $p<0.01$) as well as the velocity of perfusion increase (10.4±16.5 vs 2.6±1.5 PU.s-1, $p<0.05$) that also increased in isoglycemic phase (11.2±14.9, $p<0.05$). No other changes in MVR were observed in clamp. In control test, no MVR changes were observed at all. Generally, CAM declined at the end of both the clamp and the control test. Similar was true for lipid parameters. Fibrinogen increased slightly at the end of the hyperglycemic phase of the clamp (3.6±0.8 vs 3.4±0.9 g.l-1, $p<0.01$) and on the contrary, declined in control test (3.2±0.9 vs 3.4±1.0 g.l-1, $p<0.05$). No relationship was observed between measured parameters during both clamp and control test.
Microvascular reactivity in clamp was probably modulated by vasodilatory effect of hyperinsulinemia. Decline in CAM was caused by non-specific effect (hemodilution). No relationship was observed between acute hyperglycemia (+5.5 mmol.l-1) and microvascular reactivity in Type 1 diabetic patients.
Supported by the research project MSM0021620807 of the Ministry of Education of Czech Republic.

PDO9

LONG-TERM EFFECT OF TETRAHYDROCURCUMIN SUPPLEMENTATION ON CEREBRAL BLOOD FLOW AND ENDOTHELIAL FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Tetrahydrocurcumin (THC), a strong antioxidant is one of the major colorless metabolites of the curcumin. Antioxidants may be useful in preventing vascular diseases. Among the antioxidant agents, THC has not yet been examined in regard to its effectiveness in ameliorating cerebral blood flow impairment and endothelial dysfunction in diabetes. The objective of this study is to examine the long-term effect of THC supplementation on the cerebral microcirculation in diabetes, using streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in male Wistar Furth rats by a single intravenous injection of STZ (55 mg/kg BW). The rats were divided into control and diabetic groups either with or without THC supplementation (STZ-THC, STZ, respectively). THC was supplemented at a dose of 100mg/kg BW for 24 weeks. The cerebral microcirculation of all experimental groups was directly observed using fluorescence video microscopy. The regional cerebral blood flow (rCBF) was continuously measured using laser Doppler flowmetry. Leukocyte adhesion to endothelium was evaluated in cerebral post capillary venules by counting the number of adherent cells labeled with rhodamine 6G. In addition, functional responses of cerebral arterioles to endothelium-dependent (acetylcholine) and -independent (nitroglycerin) agonists were measured in control non-diabetic and diabetic rats with or without THC supplementation. The results demonstrated that the number density of adherent leukocytes was significantly increased in STZ-rats while rCBF was decreased compared to non-diabetic rats. In the STZ -THC rats, the rCBF was significantly greater, whereas the leukocyte adhesion was significantly less than that in the STZ rats. Furthermore, the magnitude of vasodilation to acetylcholine was significantly less in STZ-rats. Supplementation with THC significantly improved vasodilation in STZ rats. Response of pial

arterioles to nitroglycerin was similar in control, STZ and STZ-THC rats. In conclusion, long-term supplementation of THC suppressed leukocyte adhesion and thus endothelial dysfunction, associated with increase in cerebral blood-flow in diabetes. It was suggested that tetrahydroccurcumin might be a great supplemented agent for preventing diabetic cerebral microangiopathy.

PDO10

EFFECT OF WEIGHT REDUCTION ON VASCULAR FUNCTION, ERECTILE DYSFUNCTION AND PROINFLAMMATORY CYTOKINES IN PATIENTS WITH VISCERAL OBESITY

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The aim of this study was to characterize the erectile and vascular functions in men with visceral obesity (<40 years of age) without the presence of apparent organic vascular disease. In addition, we hypothesized that a 3-month weight loss program (orlistat+dynamic training+diet) will influence both erectile and vascular functions and that correlation exist between the metabolic parameters, the plasma levels of TNFalpha, adiponectin, vascular function and waist/hip ratio (W/H). According to the Erectile Dysfunction International Index (EDII) in 52 obese patients involved, we found moderate or light erectile dysfunction in 20 patients (Ob-ED), whereas in 30 patients were without ED (Ob). We have found significant differences (p<0,05) between Ob-ED and Ob patients in the following parameters: adiponectin, TNFalpha, vascular function measured with FMD, waist/hip ratio (W/H). Next we investigated the effects of weight loss in Ob-ED and Ob patients and compared them to those of healthy control group (C) (n=20 each). We have found significant weight loss after the three month program (BMI: 33,2±4 vs. 27,9±2,9 kg/m2) and significant improvement in erectile function (EDII: 17,2±2,8 vs. 25,7±2,9) in the Ob-ED group. In addition, there were significant improvement in vascular function (FMD: 4,87±0,7 vs. 5,89±1,1 %), adiponectin (4,6±2,2 vs. 7,9±3,1 mg/ml, TNFalpha (27±6,8 vs. 18,5±5,1 pg/ml), and W/H ratio (1,2+0,1 vs. 0,91+0,1) in Ob-Ed group. We suggest that visceral obesity – even in young man – could contribute to erectile dysfunction, likely via promoting the development of inflammatory vascular phenotype and dysfunction. Grants: OTKA T48376, K67984, K71591; ETT 364/2006; Hungarian Neuroimaging Foundation

PDO11

OXIDATIVE STRESS MARKERS AND THROMBOCYTE FUNCTION IN TYPE-1 AND TYPE-2 DIABETIC PATIENTS AND IN VITRO EFFECTS OF INSULIN.

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Our aim was to investigate the thrombocyte function and oxidative stress markers in the blood of type-1 (DM1), type-2 (DM2) diabetic patients and of healthy blood donors (HBD). ADP and collagen induced aggregation in platelet rich plasma (PRP) and in whole blood was determined. Prooxidant/antioxidant status and free radical (ROS) production were measured too. In vitro effect of insulin on platelet aggregation and ROS production were also investigated. ADP and collagen induced platelet aggregation in PRP of patients groups were suppressed, signing the satisfactory antiplatelet therapy. In whole blood the difference had not been observed. PMA induced ROS production were significantly higher in both patients groups, but SOD levels were significantly reduced. Insulin dose dependently reduced the collagen induced aggregation in DM-1 (p<0.01), and caused a U-shaped reduction in HBD (p<0.05), but it was ineffective in DM2. Insulin reduced ROS production in DM1 (p<0,01), and in HDB (p<0,05), but not in DM2. The levels of oxidative stress markers were the highest in DM1 patients; however the responsibility to insulin was preserved. The whole blood of DM2 patients was resistant to in vitro effects of insulin.
Study was supported by OTKA T060227, T048851 Hungarian grants.

PDO12

INFLUENCE OF AUTONOMIC NEUROPATHY ON ATHEROGENESIS IN TYPE 1 DIABETIC PATIENTS

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Cardiovascular autonomic neuropathy (CAN) is connected with increased mortality. The association of CAN and acceleration of atherosclerosis has been confirmed in type 2 diabetic patients. We were interested whether the correlation applies also for type 1 diabetic patients (Type1DM). Aim of our study was to find the association between CAN and markers of incipient atherogenesis in asymptomatic, normotensive Type1DM without cardiovascular disease and chronic complications.
21 Type1DM with CAN (group A) and 35 Type1DM without CAN (group B) were enrolled in the study. Both groups were comparable. Assessment of CAN was performed by Ewing battery. Parameters of endothelial dysfunction, chronic inflammation and microcirculation were used for incipient atherogenesis evaluation.
Results: There was no difference in any parameter of atherogenesis between groups. Following parameters of LDF were recorded in groups A/B: blood flow change after heating (%): 794 / 852 p=0.6, post-occlusion hyperemia test (time to half maximum; s) 3.4 / 3.6 p=0. 41.
Conclusion: Our data show that the presence of CAN does not correlate with acceleration of atherosclerosis in Type1DM.

Grant no. MSM 0021620814 from the Ministry of Education to the Faculty of Medicine, Charles University, Prague, Czech Republic.

PDO13

EFFECT OF ELEVATED MATERNAL AND FETAL INSULIN ON JUNCTIONAL INTEGRITY OF PERFUSED HUMAN FETO-PLACENTAL VESSELS

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Increased vascular leakage and altered junctional profiles are key features of feto-placental vessels in pregnancies complicated by Type1 insulin-dependent diabetes. Whether high insulin may contribute to the observed vascular disturbances remains unclear. The aim of this study was to establish whether perfusion of the human placenta with exogenous insulin, at diabetic maternal /fetal plasma insulin concentrations, would alter endothelial junctions and vascular leakage. Microvascular beds of normal human term placenta were perfused using a dual independent maternal and fetal perfusion system. Human recombinant insulin was added to the maternal circuit (0.2U/L; n=3) or fetal circuit (25mU/L; n=3). Control perfusions (n=3) had no added insulin. After 20 min, a 76 Mr dextran tracer (0.5mg/ml) was introduced to the fetal circuit for a further 10 min. The maternally administered insulin group showed no significant differences in staining or tracer leakage from the control. In the fetal insulin group, there was a significant ($p<0.05$) loss of junctional β-catenin, with 47% of vascular profiles exhibiting complete loss of β-catenin. Here, the percentage of vessels with tracer leakage was significantly higher than the control ($p<0.05$). Our data suggests that fetal hyperinsulinaemia may be a major contributor to the vascular dysfunction seen in the diabetic placenta.

PDO14

MOLECULAR MECHANISMS UNDERLYING ADVANCED GLYCATION END-PRODUCT INDUCED HEME OXYGENASE-1 MRNA AND PROTEIN EXPRESSION IN BOVINE AORTIC ENDOTHELIAL CELLS

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Hyperglycaemia and diabetes are associated with increased formation of advanced glycation end-products (AGE) with enhanced oxidative stress leading to the progression of diabetic vascular pathologies. The redox sensitive transcription factor Nrf2 mediates the induction of cytoprotective genes such as heme oxygenase-1 (HO-1) by binding the antioxidant response element (ARE) in the promoter region of the gene. HO-1 catabolises degradation of pro-oxidant heme to generate carbon monoxide and antioxidants biliverdin and bilirubin. The present study has investigated the molecular mechanisms by which AGE modulates superoxide generation, Nrf2 activation and HO-1 induction in bovine aortic endothelial cells (BAEC). Treatment of BAEC with AGE-BSA (100 µg ml-1) (0-24 h), but not native BSA, elicited a significant increase in superoxide generation, nuclear translocation of Nrf2 and increase in HO-1 mRNA (4-12 h) and protein (8-24 h) expression. Increased HO-1 protein expression induced by AGE-BSA was attenuated by inhibitors of NADPH oxidase and JNK signaling pathway, indicating that NADPH oxidase-derived superoxide and JNK signaling pathway may underlie AGE-induced HO-1 expression in BAEC. These results suggest that AGE-BSA may facilitate the induction of adaptive antioxidant genes in endothelial cells via the Nrf2/ARE pathway, possibly mediated via the JNK signaling pathway.

PDO15

ACUTE INSULIN RESISTANCE RESULTS IN ALTERED VASOMOTION IN RAT MUSCLE.

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Insulin is associated with both increased total blood flow and increased capillary recruitment in skeletal muscle. This study uses the peripheral vasoconstrictor, α-methylserotonin (αMT) to determine its effect on microvascular vasomotion in muscle. Heart rate, blood pressure, femoral blood flow, vascular resistance and hindleg glucose uptake were determined during control and hyperinsulinaemic euglycaemic clamp conditions in anaesthetised rats. Microvascular vasomotion in muscle was assessed by applying wavelet analysis to laser Doppler flowmetry (LDF) signals. Five frequency bands corresponding to the heart beat and respiration as well as from myogenic, neurogenic and endothelial activities were detected. Insulin infusion alone increased femoral blood flow, the absolute LDF signal strength as well as the myogenic component of vasomotion. When infused alone αMT decreased the absolute LDF signal and the myogenic component of vasomotion without affecting total muscle blood flow. Infusion of αMT before insulin blocked the effects of insulin on total blood flow, as well as LDF signal and the myogenic component of vasomotion. These data suggest that insulin action to recruit capillaries may be in part via action on the vascular smooth muscle itself. In addition, αMT while blocking capillary recruitment also blocks the direct insulin effect on the vascular smooth muscle.

PDO16

ANTI-OXIDANT EFFECT OF CURCUMIN ON DIABETES-INDUCED ENDOTHELIAL DYSFUNCTION: AN IN VIVO DETECTION USING HYDROETHIDINE

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Several studies showed that hyperglycemia-induced oxidative stress associated with PKC upregulation play roles in the pathogenesis of diabetic cardiovascular complications. In particular, the endothelial dysfunction seems to be the major target of those factors. Therefore, this study aimed to examine whether curcumin, an antioxidant, could improve diabetes-induced endothelial dysfunction via its direct effect on free radical (ROS) scavenging. Wistar Furth rats were devided into two groups: control (CON) and diabetes rats (DM, induction by 55 mg/kg streptozotocin, *i.v.*). After 6 weeks, diabetes-induced endothelial dysfunction was confirmed by Ach-induced vasodilatation. Then DM rats were further divided into three groups: untreated DM rats (DM) and DM rats daily treated with low and high doses of curcumin (30 and 300 mg/kg; DM+cur30 and DM+cur300, respectively). The results showed that at 12 weeks, both low and high doses of curcumin could improve Ach-induced vasodilatation. Especially, the *in vivo* detection of ROS using hydroethidine (HE), 5x10-6M, indicated that curcumin treated groups could reduce ROS levels significantly. Therefore, curcumin supplementation could improve diabetes-induced endothelial dysfunction partially associated with its ROS scavenging, and thereby appeared to interfere with the downstream PKC signaling pathway.
Acknowledgement: The 90th Anniversary Research Fund, Chulalongkorn University

PDO17

ALTERED ARACHIDONIC ACID METABOLISM IN PENILE ARTERIES DURING METABOLIC SYNDROME

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Diabetes mellitus is strongly associated with an increased risk of erectile dysfunction. Since abnormal reactivity is an early manifestation of vascular complications in type 2 diabetes, the aim of the current study was to investigate the vasoactive actions of arachidonic acid (AA) in penile arteries of prediabetic obese (OZR) compared to lean Zucker (LZR) rats. AA elicited relaxations in phenylephrine-precontracted arteries which were impaired in OZR (maximal responses 11 +/- 2 in OZR *vs* 21 +/- 2% in LZR, p<0.01, n=28 respectively). Inhibition of COX-1 enhanced AA relaxant effect in LZR (36 +/- 7%, p< 0.01 *vs* control), whereas selective blockade of COX-2 markedly increased relaxations to AA in both LZR (50 +/- 6, p< 0.05 *vs* control, n=7) and OZR (41 +/- 9, n=7, p< 0.05 *vs* control). Inhibition of the TXA2 receptor also enhanced AA-elicited relaxations in both LZR (37 +/- 8%, p<0.05 *vs* control, n=10) and OZR (25 +/- 6, p<0.05 *vs* control, n=10). Endothelium removal did not change AA responses in either LZR or OZR. These findings suggest that COX-1 and COX-2 both play a role in the physiological regulation of penile arteries through the balanced production of constrictor and dilator prostanoids. An enhanced COX-2-dependent production of vasoconstrictor prostaglandins occurs in OZR. Supported by grant SAF2006-09191 (Spain).

PDO18

INSULIN PERFUSION DECREASES EXPRESSION OF THE SPLICE VARIANT VEGF165B IN LARGE VESSELS OF THE HUMAN TERM CHORIONIC STEM VILLI BUT NOT IN MICROVESSELS

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In diabetic pregnancies, fetal hyperinsulinaemia may affect placental vascular development and permeability. VEGF165b, a splice variant of VEGF165a, is thought to be anti-angiogenic, but pro-permeability. Whether insulin can affect the expression of these variants in the human placenta is not known and is the aim of this study. Using normal full term placenta and a well-established perfusion system, human recombinant insulin (25mU/L) was added to the fetal circuit of the experimental group (n=3), but not to control perfusions (n=3). 76Mr dextran tracer was added to all perfusions to assess vascular leakage. In the insulin-perfused placenta the mean percentage of vessels with tracer leakage was 28%, significantly (p<0.05) higher than the 9% seen in controls. This leakage was seen in microvessels and large vessels of the insulin group. VEGF 165b was localised to the trophoblast and vascular layer of chorionic villi in both groups. However, in the insulin-perfused placentas, there were significant reduction in the percentage of large conduit vessels showing VEGF165b (p<0.05) whilst there was a global increase in VEGF165a . Our data suggests that VEGF165b may play a role in inhibiting vascular leakage in large vessels; the latter can be diminished by short duration insulin insult. Funded by ASGBI.

PDO19

ACUTE EFFECTS OF VITAMIN C ON DIABETES-INDUCED ENDOTHELIAL DYSFUNCTION IN ASSOCIATED WITH TETRAHYDROBIOPTERIN BIOAVAILABILITY

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The beneficial effects of vitamin C supplementation have been reported for improving endothelial dysfunction in diabetes, but its underlining mechanism(s) remains unclear. To investigate further on the *acute* effect of vitamin C administration, the animals were divided into two majors groups: control (CON) and diabetic groups (DM; iv. 50 mg/kg BW streptozotocin). The 10^{-5} M acetylcholine (Ach)-induced vasodilatation was assessed after each following experimental protocol for both groups: 1) Baseline diameter; 30-min Krebs-Ringer perfusion, 2) +Vit.C; 1-min 2.6 mM vitamin C administration, 3) +DAHP; 30-min tetrahydrobiopterin (BH_4) antagonist (20mM, 2,4-diamino-6-hydroxypyrimidine (DAHP), Sigma-Aldrich Co., USA), 4) +DAHP+Vit.C; 1-min 2.6 mM vitamin C administration after 30 min DAHP, 5) +DAHP+BH_4; 20-min BH_4 donor (0.1 mM) after 10-min DAHP, and 6) DAHP+BH_4+Vit.C; 10-min DAHP followed by 20-min BH_4 donor and 1-min vitamin C. The results

showed that the DAHP significantly reduced Ach-induced vasodilatation in both CON and DM ($p<0.001$). Interestingly, in both DAHP-treated CON and DM groups, the Ach-vascular responses were improved by Vit.C, BH_4 donor, and the combined Vit.C and BH_4 administrations ($p<0.001$). Our findings suggested that the acute effect of vitamin C on diabetes-induced endothelial dysfunction could be improved partially involved intracellular BH_4 content and subsequent enhancement of eNOS activity. Acknowledgement: The 90th Anniversary Research Fund, Chulalongkorn University

PDO20

IN VIVO ASSESSMENT OF MICROVASCULAR PERFUSION AND GLUCOSE UPTAKE: EFFECT OF ACUTE MICROSPHERE EMBOLISM

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Insulin possesses hemodynamic activity that enhances its delivery to myocytes, which in turn improves its metabolic action. To better understand this interaction, we examined the effect of decreased muscle microvascular perfusion on insulin sensitivity by partial blockage of the microvascular bed with 15 microm diameter latex microspheres. *In vivo* experiments were carried out in anaesthetized rats. A bolus of microspheres (1.5, 3 or 7 million) was injected into the hindleg via the femoral artery by retrograde infusion in the epigastric artery of a normal rat. The contralateral leg was used as control. The local effect of microsphere embolism on the insulin sensitivity was assessed during a euglycemic hyperinsulinemic clamp (10 mU.min-1.kg-1 x 2h). None of the doses of microspheres had any effect on insulin-mediated increases in femoral blood flow. However, microvascular perfusion, assessed by the metabolism of infused 1-methyl xanthine, was not affected by injection of 1.5 million microspheres but decreased by 50% at the higher doses. Similarly, glucose uptake in the treated leg showed a decrease of 50% with the injection of 7 million microspheres. In conclusion, microsphere embolism diminished microvascular perfusion and insulin-mediated glucose uptake, providing a microvascular-derived model of insulin resistance, which can be used for chronic studies.

PDO21

ENHANCED NEOINTIMAL HYPERPLASIA AND CAROTID ARTERY REMODELING IN A170/P62 DEFICIENT OBESE MICE

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Signal adaptor protein A170/p62 plays an important role in membrane receptor mediated signal transduction. Since A170 deficient (KO) mice exhibit mature onset obesity, we examined vascular integrity in A170 KO mice. We isolated aortic smooth muscle cells (SMCs) and found that A170 deficient SMCs proliferate much faster and attain 2-3 fold higher cell densities than the wild type SMCs upon serum stimulation *in vitro*. Moreover, serum stimulation led to an enhanced and sustained activation of p38 MAPK in A170-KO compared to wild type SMCs. Inhibitors of p38 and ERK1/2 suppressed SMC proliferation in A170-KO and WT. Our findings suggest that A170 suppresses proliferation of vascular SMCs at least partly through down-regulation of p38 MAPK activation in response to growth stimulations *in vitro*. To further characterize the function of A170 in the arterial wall *in vivo*, we examined the effects of complete ligation of the right common carotid artery, i.e. a blood flow cessation model, on vascular remodeling. We found that A170-KO mice exhibited an enhanced thickening of the medial layer after 2-3 weeks ligation, suggesting that A170 affords cytoprotection against neointimal hyperplasia and carotid artery remodeling after vascular injury.
Supported by Great Britain Sasakawa Foundation

PDO22

DIFFERENTIAL STRUCTURAL AND FUNCTIONAL CHANGES OF PENILE AND CORONARY ARTERIES IN METABOLIC SYNDROME

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Erectile dysfunction is considered an early sign of systemic endothelial dysfunction and has been proposed as a potential marker for silent coronary artery disease in type 2 diabetes mellitus. The aim of the present study was to evaluate the structural and functional changes in penile (PA) and coronary (CA) arteries of prediabetic obese (OZR) compared to lean Zucker (LZR) rats by means of *in vitro* microvascular myographs and morphometric analysis of stained arteries. Internal diameter was reduced and wall-to-lumen ratio increased in PA from OZR. Acetylcholine-elicited relaxations were severely impaired in PA but not CA from OZR although endothelial nitric oxide (NO) synthase expression was unchanged. Contractions to noradrenaline and serotonin were augmented in PA and CA, respectively, from OZR. Blockade of NO synthase markedly reduced endothelium-dependent relaxations in PA and CA, and potentiated noradrenaline and 5-HT contractions in arteries from LZR but not from OZR. The NO-mediated vasodilator response to the phosphodiesterase 5

inhibitor, sildenafil, was attenuated in both PA and CA from OZR. The present results demonstrate vascular inward remodelling in PA and a differential impairment of the basal and stimulated NO-mediated endothelial responses in PA and CA from animals with *Metabolic Syndrome*. The severity of the altered structure and function in PA might anticipate the functional impairment of the more preserved coronary vascular bed. Supported by grant SAF2006-09191 and SF 2006-02376 (Spain).

PDO23

EFFECTS OF CURCUMIN AND CURCUMIN ANALOGS ON DIABETES INDUCED ENDOTHELIAL DYSFUNCTION

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This study was aimed to evaluate the effects of curcumin and its analogs, tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin, whether they could protect the functions of endothelial cells in diabetes mellitus. Animals were divided into six groups: control (CON), diabetes (DM, iv. streptozotocin (STZ) 55 mg/kgBW), DM treated with curcumin (CUR, 300 mg/kgBW/day), DM treated with tetrahydrocurcumin (THC, 100 mg/kgBW/day), DM treated with hexahydrocurcumin (HHC, 100 mg/kgBW/day), and DM treated with octahydrocurcumin (OHC, 100 mg/kgBW/day). All treatments were started at 10 days after the STZ-injection. At 8th week, mesenteric microcirculation was observed for leukocytes adhesion in postcapillary venules and microvessels were labeled with FITC-dextran to study arterioles responses to acetylcholine 10-5 M (Ach 10-5M) and sodium nitroprusside 10-5 M (SNP 10-5M). At the end of experiment, blood samples and livers were collected for biochemical and oxidative stress analyses, respectively. The results demonstrated that numbers of leukocytes adhesion in CUR and THC groups were significantly decreased as compared to DM group ($p<0.05$). The responses of mesenteric arterioles to Ach 10-5M in every groups of curcumin-analogs treatment were increased as compared to DM group ($p<0.05$). Therefore, our findings suggested that curcumin and their analogs could protect diabetes-induced endothelial dysfunction.
Acknowledgement: National Research Council of Thailand

PDO24

EFFECT OF VITAMIN C ADMINISTRATION ON NITRIC OXIDE LEVELS IN DIABETIC RATS: AN IN VIVO DETECTION USING DAF-2DA

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The present study aimed to examine the *direct* effect of vitamin C administration on diabetes-induced endothelial dysfunction in associated with nitric oxide (NO) bioavailability using the novel NO-sensitive fluorescent indicator. The experiment was performed by using control (CON) (n=12, male Spraque-Dawley rat; weighing 200-250g), and streptozotocin-induced diabetic rats (DM) (n=12, iv. injection of STZ (50 mg/kg BW)). To determine the *in-vivo* effect of vitamin C administration on NO bioavailability, the NO-sensitive fluorescent dye, 4,5-diaminofluorescein-diacetate (DAF-2DA, 3 μM, Daiichi Pure Chemicals Co. Ltd.), was used. At 20 minutes after 2.6mM-vitamin C co-perfusion with DAF-2DA, 10uM acetylcholine (Ach), and Krebs Ringer buffer (0.5 ml/min), the changes of DAF-2T fluorescence intensities along the mesenteric arteriolar vascular walls were analyzed by Image Pro-Plus Software (Media Cybernatics, Inc, USA). The results indicated that the administration of vitamin C could increase the vasodilatation in both control (23.49 ± 3.03%) and diabetic groups (15.05 ± 3.22%). Moreover, 20-min after 2.6 mM vitamin C administration, the DAF-2T fluorescence intensities were significant increase in both groups of control (235.43 ± 28.00 %) and DM (147.21 ± 21.98%), as compared to their based-line values ($p<0.001$). It may conclude that vitamin C could reverse diabetes-induced endothelial dysfunction via enhancing NO bioavailability.
Acknowledgement: Ratchdapisek-Sompoch Research Fund, Faculty of Medicine, Chulalongkorn University

Wednesday, August 27th
Hypertension
Poster Session - PHT

PHT1

GENE EXPRESSION OF ANGIOGENIC FACTORS IN MUSCLE TISSUE DURING AGE-RELATED DEVELOPMENT OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Essential hypertension has been associated with capillary rarefaction but little is known about the cellular mechanisms underlying this process. We examined the gene expression of angiogenic factors during age-related development of hypertension in spontaneously hypertensive rats (SHR). Wild-type Wistar Kyoto (WKY) rats served as controls. White gastrocnemius muscle was obtained and blood pressure was monitored at 5, 10 and 15 weeks of age. In the SHR group, systolic blood pressure increased from 5 to 10 and 15 weeks of age and the levels were higher than in the WKY group at 10 and 15 weeks (~70%; $P<0.05$). The mRNA content for MMP-2 was overall lower ($P<0.05$) in SHR compared to WKY. VEGF mRNA increased ($p<0.05$) from 5 to 10 weeks in SHR and there was a general increase ($P<0.05$) in the VEGF receptor flt-1. There was a trend for a lower content of eNOS and CYP 2C11 in the SHR than in WKY group. There were no alterations in the mRNA content of KDR, AMP 5'- nucleotidase, or Cytochrome P450 4A. The results show that the age-related development of hypertension from 5 to 15 weeks in SHR rats is not associated with major changes in mRNA content of the herein included angiogenic factors.
Support: Danish Medical Research Council, Novo Nordisk Foundation and Lundbeck Foundation

PHT2

WHOLE BODY BLOOD FLOW AUTOREGULATION AND HYPERTENSION: INSIGHTS FROM COMPUTER SIMULATIONS WITH GUYTON'S MODEL

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Computer simulations offer a unique way to test the role of whole body blood flow autoregulation (WBFAR) in the pathogenesis of salt-loading hypertension since it is not possible to block WBFAR experimentally. Using Guyton's large circulatory model, we analyzed, in the presence or absence of WBFAR, the hemodynamic and fluid volume changes in a classical model of volume-loading hypertension induced by reducing renal mass to 30% of normal and increasing salt intake by 6-fold. In the presence of WBFAR, the development of hypertension (+30 mmHg) was characterized by an initial increase in cardiac output (CO) by ~30% followed by a secondary increase in vascular resistance (+25%). In the absence of WBFAR, the same simulation led to similar long-term increases in blood pressure (BP). However, a nearly two-fold increase in CO and an increase in extracellular fluid volume by more than 30% were observed whereas vascular resistance decreased by 30% owing to the mechanical distension of blood vessels by the increased BP. From the simulations one may conclude that autoregulation limits the amount of fluid retention required to increase BP in order to achieve salt balance during salt-loading, but does not affect the final level of BP.

PHT3

ANGIOTENSIN II RECEPTOR 1 BLOCKER CANDESARTAN REDUCES LIPOPROTEIN-INDUCED AT_1 EXPRESSION AND OXIDATIVE STRESS

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Oxidized low-density-lipoprotein (oxLDL) and angiotensin II (Ang-2) can increase formation of reactive oxygen species in endothelial cells. However, interactions of both risk factors and impact of AT_1 receptor blockade on lipoprotein-induced oxidative stress and endothelial function is currently not well understood. In this project, impact of native and oxidized LDL (nLDL, oxLDL) on Ang-2 receptor expression and formation of reactive oxygen species was analyzed in primary cultures of human umbilical arterial endothelial cells (HUAEC). Native and oxLDL induced already after 1 h endothelial AT_1 and AT_2 receptor expression. Both lipoproteins increased intracellular AT_1 receptor immunofluorescence. Native LDL and oxLDL activated MEK/ERK and p38 MAPK pathways. Furthermore, oxLDL induced the oxLDL receptor LOX-1 expression. Induction of both Ang-2 receptors by oxLDL could be reduced by preincubation with AT_1 receptor antagonist candesartan. AT_1 receptor blockade prevented lipoprotein-induced oxidative stress like SOD in HUAEC. In murine aortic rings, oxLDL-induced vascular superoxide anion formation was reduced by AT_1 blockade. AT_1 blockade prevented impaired endothelial function in response to oxLDL. In conclusion, augmented vascular oxidative stress and endothelial dysfunction in response

to lipoproteins involves induction of the AT_1 receptor. Our data suggest a novel vasoprotective mechanism of AT_1 receptor blockade in hypercholesterolemia. Supported by the German Federal Ministry of Education and Research and AstraZeneca.

PHT4

ENHANCED EXPRESSION OF TREK-1 K^+-CHANNELS IN ENDOTHELIUM OF KCA3.1-KO MICE AND THE SPONTANEOUSLY HYPERTENSIVE RAT

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The two-pore-domain potassium (K_{2P}) channels TREK-1 and TREK-2 are mechanosensitive and polyunsaturated fatty acid-activated channels. Herein, we characterized the expression of TREK-1/2 channels in the endothelium and their function in endothelium-dependent vasodilation. Moreover, we investigated whether TREK-1/2 channels contribute to altered endothelial vasodilator responses in mildly hypertensive K_{Ca}3.1-KO mice and the spontaneously hypertensive rat (SHR). mRNA-expression of TREK-1 and TREK-2 in the endothelium was analyzed by single-cell-RT-PCR in carotid artery endothelial cells (CAEC). The channel function was studied using the patch-clamp-technique and pressure-myography in carotid arteries (CA). CAEC from K_{Ca}3.1-KOs and wild-types expressed mRNA coding for TREK-1, but not for TREK-2. TREK-1 currents in CAEC were elicited by hypo-osmotic challenge, intracellular acidosis, arachidonic acid (AA), isoflurane, and acetylcholine (ACh). In CAEC from K_{Ca}3.1-KOs, TREK-1 mRNA-expression and functions was greatly enhanced as compared to controls. Likewise, TREK-1-mediated and EDHF-type vasodilator responses to TREK-1 stimuli were increased in CA of K_{Ca}3.1-KOs. Similarly, enhanced endothelial TREK-1 gene expression and TREK-1 channel functions were evident in SHR. In conclusion, TREK-1 channels are functionally expressed in rodent CAEC and contribute to isoflurane and ACh-induced EDHF-type vasodilator responses. Our results suggest that up-regulation of endothelial TREK-1 may provide an alternative vasodilatory reserve in hypertension.

PHT5

ESSENTIAL HYPERTENSION: ELASTASE, NITRIC OXIDE METABOLITES AND OXIDATIVE STATUS

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In essential hypertension (EH) few are the data about the behaviour of plasma elastase and contrasting are those concerning the end products of nitric oxide (nitrite + nitrate: NOx). As regards the oxidative status, several reports point out an increase in lipid and proteic peroxidation, while the total antioxidant status (TAS) seems almost always reduced. We examined plasma elastase, NOx and oxidative status (thiobarbituric acid-reactive substances –TBARS- and TAS) in a group of 25 subjects with untreated mild essential hypertension. In hypertensives in comparison with normal controls we found a significant increase of NOx (control subjects: 28.36 +/- 18.36 microM; EH: 48.78 +/- 23.17 microM; $p < 0.001$) and TBARS (control subjects: 5.75 +/- 1.21 nmol/ml, EH: 12.00 +/- 1.69 nmol/ml; $p < 0.001$) without any variation of elastase and TAS. A significant correlation was only observed between elastase and day-time systolic and diastolic pressure and between elastase and 24-h systolic and diastolic pressure. This data might support the use of molecules able to influence the oxidative status. The trend of NOx is likely dependent on the macrophage iNOS overproduction, but it might have a positive role because nitrite and nitrate can be probably reduced to NO once again.

PHT6

EFFECTS OF ANTIHYPERTENSIVE DRUGS FROM DIFFERENT PHARMACOLOGICAL CLASSES ON CAPILLARY RAREFACTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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We investigated whether chronic antihypertensive treatment reverse capillary rarefaction in spontaneously hypertensive rats (SHR), using Wistar normotensive rats as controls (WKY). Functional capillary density (FCD) was studied using fluorescence intravital videomicroscopy. In untreated rats, FCD was lower in SHR skeletal muscle and ear skin. Histological analysis showed that SHR had lower capillary-to-fiber ratio in the skeletal muscle and lower capillary volume density-to-fiber volume density ratio in the left ventricle. The animals were treated during 28 days with the angiotensin-converting enzyme inhibitor enalapril, or the AT_1 receptor antagonist losartan, or the ß-blocker atenolol or the calcium channel blocker nifedipine, resulting in similar reductions in systolic blood pressure. Atenolol did not induce any change in FCD of SHR. Losartan and nifedipine completely reversed functional capillary rarefaction in both muscle and cutaneous tissues while enalapril significantly increased FCD only in the skin. The skeletal muscle capillary-to-fiber ratio was normalized by enalapril, losartan and nifedipine. Treatments with enalapril or losartan normalized the cardiac structural capillary rarefaction of SHRs while atenolol and nifedipine had no effect. Our results suggest that different pharmacological classes of antihypertensive drugs with similar effect on blood pressure differ in terms of their effect on the microcirculation. Supported by CNPq, FAPERJ and FIOCRUZ.

Wednesday, August 27th
Endothelial cell biology and function
Poster Session – PEC

PEC1

LIGHT EFFECT ON NITRIC OXIDE QUANTIFICATION IN VITRO USING DAF-2

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Quantification of nitric oxide (NO) in the microcirculation has been carried out primarily using microelectrodes with their inherent spatial and temporal limitations. An alternative approach to measuring NO, both in vitro and in vivo, uses the fluorescent indicator DAF-2 that forms the strongly fluorescent product DAF-2T following its reaction with an auto-oxidation product of NO. In this study we report that the rate of change of DAF-2T fluorescence is proportional to $[NO]^2$, as predicted by the reaction kinetics of NO and DAF-2 in the presence of O_2. We also investigated the effect of light exposure on fluorescence photo-activation and photobleaching. The conversion of DAF-2 to DAF-2T was evaluated in mixtures of 10 μM DAF-2 with NO ranging from 100 to 1200 nM. Sequences of fluorescence images were acquired with a digital camera. Light intensities normally used in microscopic measurements induced strong photobleaching when the interval between consecutive light exposures was shorter than 10 sec. Photo-activation, not related to DAF-2T, was observed if the area illuminated was large. To minimize the light exposure artifact, the incident light must be restricted to a small area and the measurements limited in time. The non-linear relationship between DAF-2T fluorescence and [NO] was adequately described by the chemical kinetic model when the PO_2 of the mixture was taken into account. These results will be used to quantify NO in the microcirculation in vivo. Support by NIH grants HL 18292 and HL 079087.

PEC2

REPEATED PASSIVE LEG MOVEMENT ENHANCES INTERSTITIAL VEGF PROTEIN, ENDOTHELIAL CELL PROLIFERATION, AND MMP-2 AND MMP-9 mRNA CONTENT IN HUMAN SKELETAL MUSCLE.

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This study examined the effect of training by passive leg movement, which enhances blood flow and passive stretch, on muscle capillarization, endothelial cell proliferation, VEGF protein, and mRNA levels. The model used was 90 min of passive one-leg knee extensions 4 times/week for 4 weeks. Muscle biopsies were obtained during training and muscle microdialysate was collected during passive and active movement in the trained (TL) and untrained (UTL) leg. Interstitial VEGF protein levels increased ($p < 0.05$) with passive and active movement above rest with no difference between groups. Microdialysate obtained during both passive and active leg movement in UTL had a ~2.4-fold higher ($P<0.05$) proliferative effect than resting microdialysate. The proliferative effect was similar in TL and UTL. There was a 2-fold greater ($p < 0.05$) number of Ki-67-positive cells in TL compared UTL at week 2. There was a general increase (~7%, ~100% and ~500%, respectively; $p < 0.05$) in capillaries per mm^2, MMP-2 and MMP-9 mRNA content from Pre to 4 weeks of training with no difference between groups. The results show that a period of repeated bouts of passive movement enhances angiogenic factors and endothelial cell proliferation in skeletal muscle probably via shear stress and passive stretch. The study was supported by The Danish Ministry of Culture (Kulturministeriets Udvalg for Idrætsforskning).

PEC3

INHIBITORY INTERACTION OF ENDOGENOUS PLASMA MEMBRANE CALCIUM/CALMODULIN ATPASE ISOFORM 2 WITH ENDOTHELIAL NITRIC OXIDE SYNTHASE IN PRIMARY ENDOTHELIAL CELLS

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The ability of the endothelium to synthesise and release nitric oxide (NO) plays a major role in the regulation of cardiovascular physiology. In endothelial cells NO is synthesised by the constitutively expressed endothelial form of nitric oxide synthase (eNOS). eNOS activity is regulated at several levels, including intracellular calcium/calmodulin concentrations and interaction with partner proteins. We have detected a novel interaction between endogenous eNOS and the Plasma Membrane Calcium ATPase (PMCA) in primary HUVEC endothelial cells. PMCA proteins function as calcium pumps removing calcium from the cell to the extracellular milieu, and emerging evidence suggests a novel role for these enzymes as regulators of calcium/calmodulin-dependent signal transduction pathways. PMCA isoforms 1, 2 and 4 are expressed in HUVEC cells. Immunoprecipitation experiments of endogenous and ectopically expressed PMCA2 have revealed a strong interaction between PMCA2 and eNOS. The interaction domains mapped to the amino acid region 462-684 of PMCA2 and to region 735-934 of eNOS. At the functional level the PMCA2/eNOS interaction results in inhibition of eNOS activity and subsequent NO production. Our results suggest a novel role for PMCA2 as a regulator of NO production in endothelial cells, and, therefore, of NO-mediated physiological processes such as angiogenesis and platelet aggregation.

PEC4

PRE-INCUBATION WITH ESTROGEN MODULATES ENDOTHELIUM-DEPENDENT REACTIVITY IN SMALL ARTERIES FROM OFFSPRING OF PROTEIN-RESTRICTED DAMS GIVEN STATIN TREATMENT

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Endothelial dysfunction induced in male rat offspring by maternal protein restriction can be prevented by statin treatment, but dysfunction in females is not consistent. This study aimed to determine if pre-treating mesenteric vessels with estrogen (E2) could highlight any differences in females and further gender differences.
Pregnant Wistar rats were fed control or protein-restricted (PR) diet throughout gestation. Post-weaning, a subset of PR offspring were given atorvastatin (PRS; 10 mg/kg/day) until 475 days old, when small mesenteric arteries were assessed by wire myography.
Vasodilatation to E2 and acetylcholine (ACh) +/- E2 30min pre-incubation was observed. Analysis was performed by two-way ANOVA with Bonferroni *post-hoc* analysis.
Responses to E2 were similar in all groups in both males and females and were insensitive to L-NAME (100microM) blockade. In females, ACh relaxation was similar in all groups, while in males it was attenuated in PR but not PRS ($p<0.05$). Pre-incubation with E2 had no effect on responses to ACh in male offspring; however, in females responses were attenuated in PR ($p<0.05$) and enhanced in the controls and PRS ($p<0.05$).
These data demonstrate gender dimorphism for endothelial dysfunction following maternal protein restriction.

PEC5

CIRCULATING ENDOTHELIAL PROGENITOR CELLS (EPCS) IN PATIENTS UNDERGOING ALLOGENEIC TRANSPLANTATION (ALLO-SCT)

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EPCs can influence physiological and pathological formation of blood vessels. We have previously demonstrated that patients with small vessels disorders have decreased numbers of EPCs. Patients undergoing alloSCT might have endothelial damage as well. We have performed cultivation assay (CFU-Hill) and flow-cytometry measurements to determine EPC counts in 17 patients before alloSCT. Their results have been compared with two control populations: 17 untreated patients with hematological malignities and 36 healthy volunteers. The age medians or leukocyte counts between these three groups did not differ. Both patients before alloSCT and untreated hematological patients had significantly decreased numbers of EPCs compared to healthy volunteers. We did not observe any significant difference between untreated hematological patients and patients undergoing alloSCT. After alloSCT, further decrease in CFU-Hill colonies was observed and their number did not return to pre-transplant number up to one year after transplant. There was no correlation between CFU-Hill number and number of CD34+/CD133+ or CD34+/KDR+ cells measured by flow cytometry. The decrease of EPCs in transplanted patients is probably not only related to cytotoxic treatment but also to underlying hematological disease. This decrease can lead to higher risk of cardiovascular disorders, described in patients undergoing alloSCT.
Supported by: IGA MZ CR 8754/3, GAUK 33/2006/C/1.LF, MSM 0021620808

PEC6

ROLE OF THE ENDOTHELIUM ON VASOMOTION: A THEORETICAL STUDY

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It is well-known that cyclic variations of the arterial diameter (vasomotion) are induced by synchronous calcium oscillations of smooth muscle cells (SMCs). However, experimental observations about the role of the endothelium on vasomotion are contradictory. We propose a theoretical model of the calcium dynamics of a population of SMCs and endothelial cells connected through homocellular and heterocellular gap junctions via electrical, calcium and inositol 1,4,5-trisphosphate coupling. We show that SMCs may be in a low calcium level steady state, in an oscillatory domain, or in a high calcium level steady state, depending on the vasoconstrictor concentration. In the oscillatory domain, a population of coupled SMCs exhibits synchronous calcium oscillations. Outside this domain, with the same coupling coefficients, SMCs present asynchronous calcium flashes and fluctuations resulting from stochastic opening of channels. We show that the endothelium derived factors increase the range of vasoconstrictor concentrations for which SMCs are in the oscillatory domain. The main effect of the endothelium is to decrease the mean calcium level in SMCs. As a consequence, the endothelium can induce a transition from the steady state to the oscillatory domain, and vice versa. These two types of transitions explain how the endothelium can induce or abolish vasomotion.

PEC7

EFFECT OF A SELECTIVE PHOSPHODIESTERASE-5-INHIBITOR ON ENDOTHELIAL DYSFUNCTION INDUCED BY PEROXYNITRITE IN RAT AORTA

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Reactive oxygen species (e.g. peroxynitrite) induce oxidative stress and DNA injury leading to endothelial dysfunction. Elevated $[cGMP]_{ic.}$ may contribute to an effective cytoprotection against nitro-oxidative stress. We investigated the effects of vardenafil, an inhibitor of phosphodiesterase-5 on endothelial dysfunction induced by peroxynitrite.

In organ bath experiments, we investigated the endothelium-dependent (acetylcholine) and -independent (sodium nitroprusside, SNP) vasorelaxation of isolated aortic rings of rats. Endothelial dysfunction was induced by peroxynitrite (200 µM). In the treatment groups, rats received vardenafil (5, 30 or 300 µg/kg, iv. 10min before removal of the aorta).

Exposure to peroxynitrite resulted in an impairment of endothelium-dependent vasorelaxation of aortic rings (maximal relaxation (R_{max}) to acetylcholine: 92.3+/-1.1% control vs. 58.7+/-3.6% peroxynitrite). Vardenafil-pretreatment led to an improvement of endothelial function (R_{max} to acetylcholine: 58.7+/-3.6% vs. 85.1+/-2.0% vs. 77.3+/-4.0% vs. 71.3+/-5.9%; 0, 5, 30 and 300 µg/kg vardenafil+peroxynitrite respectively). A moderate shift of the SNP concentration-response curves (indicative of the endothelium-independent vasorelaxation) could be seen after vardenafil-pretreatment without any alterations of the R_{max}.

Our results support the view that impairment of the intracellular cGMP-signaling play a role in the pathogenesis of the endothelial dysfunction induced by peroxynitrite, which can effectively be reversed by acute phosphodiesterase-5-inhibition.

PEC8

FUNCTIONAL ENAC CHANNELS EXPRESSED IN ENDOTHELIAL CELLS: A NEW CANDIDATE IN ENDOTHELIAL CELLS TO MEDIATE SHEAR FORCE

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Endothelial cells (ECs) form a unique signal-transducing surface in the vascular system and are regulated not only by circulating hormones but also by mechanical stresses such as shear force. Ion channels in ECs can signal rapid changes in response to shear forces and are involved in controlling EC permeability, proliferation, and angiogenesis. As a potential mechanical sensor, the α-subunit of epithelial sodium channel (ENaC) was revealed to be present in cultured human ECs and intact ECs from a variety of rat arteries. In either inside-out or outside-out current recording, currents with conductance of 4.76 pS were detected and were sensitive to micro-molar amiloride in human dermal microvascular endothelial cells (HMEC), primary cultured human dermal microvascular endothelial cells (pHMEC) and human umbilical cord vein endothelial cells (HUVEC). These currents were carried by Na^+ and replacement of low extracellular Na^+ caused a right shift of I-V curve. In the cultured intact ECs, ENaC did not mediate Na^+ current because the ENaC activity was greatly inhibited by cellular molecules such as 11, 12-EET. In the outside-out configuration, laminar flow significantly directly enhanced ENaC opening probability, suggesting a potential role of ENaC in mediating shear force signalling events. Taken together, α subunit of ENaC is predominantly expressed in ECs, but the ENaC activity is strongly inhibited by cellular molecules. When inhibition was removed, ENaC could sense flow directly by change of channel open probability and mediate Na^+ entry via channels, resulting in membrane depolarization. In co-ordination with other channels and transporters, ENaC might affect $[K^+]_o$ in smooth muscle and release of ATP, and involve in the regulation of vascular tone. As a novel candidate in sensing shear force, ENaC might play an important role in vascular biology.

PEC9

ROLE OF THE ENDOTHELIUM ON VASOMOTION: AN EXPERIMENTAL STUDY

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Synchronized activity of smooth muscle cells of the arterial wall induces cyclic oscillations of the arterial diameter which is call vasomotion. The role of the endothelium on vasomotion was contradictory in the literature. To understand how the endothelium may abolish vasomotion in certain cases and promote it in others, we analyze the role of the endothelium using rat mesenteric arterial strips stimulated with phenylephrine (PE). Our results show that vasomotion could be obtained in presence or absence of the endothelium, but not at the same PE concentration. In absence of endothelium, the PE concentration needed to induce vasomotion is lower and the rhythmic contractions and relaxations of the strips occur for a narrower range of PE concentrations. By blocking independently nitric oxide or the endothelium-derived hyperpolarizing factor, we demonstrate that these two principal endothelium-derived relaxing products may either induce or abolish vasomotion. On the one hand, when the strip is tonically contracted in a non-oscillating state, an endothelium-derived relaxation may induce vasomotion. On the other hand, if the strip displays vasomotion with a medium mean contraction, a relaxation may induce a transition to a non-oscillating state with a smaller contraction.

PEC10

EXPRESSION OF CONNEXIN 40 IN ENDOTHEIAL CELLS IS INCREASED BY SHEAR STRESS VIA A PI3-KINASE DEPENDENT PATHWAY

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Endothelial connexin 40 (Cx40) is crucial for upstream conduction of vasomotor responses. These conducted responses might be regulated by shear stress acting on endothelial cells, since shear stress is known to influence the expression and function of connexins. Therefore, we investigated if and how shear stress regulates endothelial Cx40. Confluent human umbilical vein endothelial cells (HUVEC) were exposed to defined shear stress using a cone and plate apparatus or kept under static control conditions both in the presence or absence of PI3-kinase inhibitor LY 294002, Akt-inhibitor IV, NOS-inhibitor L-NAME or its inactive analogue D-NAME. Cx40 expression was measured by quantitative real time RT-PCR and Western Blotting. mRNA expression of Cx40 increased transiently with the shear stress applied, while the amount of Cx40 protein was threefold enhanced at 24 h of shear stress. PI3-kinase inhibitor LY 294002 and Akt-inhibitor IV dramatically reduced both, Cx40 mRNA and protein expression under static and dynamic conditions. NOS-inhibitors slightly increased shear stress-dependent up-regulation of Cx40. In conclusion, shear stress-dependent up-regulation of Cx40 in endothelial cells via the PI3-Kinase pathway might contribute to adapt the conduction of vasomotor responses to flow conditions.

Wednesday, August 27th
Permeability and glycocalyx/endothelial surface layer
Poster Session - PPE

PPE1

EFFECTS OF METFORMIN ON ENDOTHELIAL GLYCOCALYX PROPERTIES IN DB/DB MICE

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The aim of this study was to determine the effects of metformin on glycocalyx exclusion and barrier properties in insulin resistant mice. Db/db mice treated with (MET) and without metformin (CON) for two weeks were infused at the day of experiment with FITC-labeled 70 kDa dextrans (D70), which have limited access to the glycocalyx, and Texas Red-labeled 40 kDa dextrans (D40), which have full access to the glycocalyx. The systemic glycocalyx volume (Vg) was determined from the difference in distribution volume of D40 and D70, while glycocalyx barrier properties were determined from the clearance exponent of D70 compared to D40 (CeD70/D40). The dry to wet weight ratio (dw/ww) was measured in the heart and kidneys. Vg was 0.009 ± 0.068 ml in CON and increased to 0.183 ± 0.127 ml in MET ($P<0.05$). CeD70/D40 was 0.619 ± 0.026 in CON and decreased to 0.530 ± 0.026 in MET ($P<0.05$). There was less dehydration of organs in MET, dw/ww was 0.257 ± 0.034 in MET compared to 0.286 ± 0.0028 in CON ($p<0.05$). In conclusion, treatment with metformin improves glycocalyx exclusion and barrier properties in db/db mice. This improvement might underlie the observed restoration of the fluid balance in these mice. Support by Dutch Diabetes Research Foundation grant 2006.00.027

PPE2

HYALURONIDASE TREATMENT OF THE ENDOTHELIAL GLYCOCALYX IMPAIRS ADENOSINE MEDIATED RECRUITMENT OF CORONARY VOLUME

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It is hypothesized that coronary hyperemia by adenosine is accompanied by increased access of circulating blood into the endothelial glycocalyx (EG). In the present study, the effect of glycocalyx degradation by hyaluronidase on adenosine mediated coronary volume recruitment was assessed. Labelled 2000kDa dextrans (Dex) and red blood cells (RBC) were infused as a bolus in the left main coronary artery in open-chest anesthetized goats using a perfusion system. Blood samples were collected from the great cardiac vein every 0.5-1 second. Coronary blood volume ($V_{cor}=V_{Dex}+V_{RBC}$) was calculated by multiplying mean transit time of each tracer with simultaneously measured coronary plasma and RBC flow. This measurement was done in control (con) and during adenosine (ado), before and after hyaluronidase treatment. Adenosine-induced coronary volume recruitment ($V_{cor,ado}/V_{cor,con}$) and conductance (C_{ado}/C_{con}) were calculated; before hyaluronidase $V_{cor,ado}/V_{cor,con}$ was 1.7 +/- 0.2 and C_{ado}/C_{con} was 2.5 +/- 0.3. After hyaluronidase $V_{cor,ado}/V_{cor,con}$ was reduced to 1.2 +/- 0.2 ($P<0.05$), while C_{ado}/C_{con} was not affected (2.3 +/- 0.2, N.S.). These data demonstrate that enzymatic degradation of the coronary EG impairs the ability of adenosine to recruit coronary vascular volume. It is concluded that recruitment of EG volume contributes to optimal matching of hyperemic coronary flow and volume. Supported by NHF grants 2005T037 and 2003B181.

PPE3

EFFECTS OF ELEVATED LEVELS OF FIBRINOGEN IN MICROCIRCULATION

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We previously showed that increased content of fibrinogen causes microvascular constriction (AJP,2005;288(3):H1257) and enhances endothelial cell (EC) layer permeability (Mol.Cell.Biochem.2008;307(1-2):13). We tested the hypotheses that at pathologically high levels, fibrinogen binding to ECs leads to enhanced production of endothlin-1 and affects tight-junction proteins. Confluent rat ECs were treated with fibrinogen (2or4mg/ml), fibrinogen (4mg/ml) with BQ-788, or medium alone. The content of formed endotehlin-1 was measured using an endotehlin-1-specific ELISA. Fibrinogen-induced exocytosis of Weible-Palade bodies (WPbs) was observed by confocal microscopy, activation of ERK signaling was assessed by detecting the phosphorylation of ERK1/2, and EC layer permeability was assessed by measuring transendothelial electrical resistance. Fibrinogen binding to ECs caused a dose-dependent increase in endothelin-1 production, enhanced WPb exocytosis, and increased ERK1/2 phosphorylation, which was diminished by BQ-788. Fibrinogen-induced EC layer permeability was correlated with altered expression of the tight-junction proteins. Our results suggest that fibrinogen-induced vasoconstriction is mediated through increased formation of endotehlin-1, and alterations in tight-junction proteins are involved in fibrinogen-induced EC layer permeability. These mechanisms point to a greater role of fibrinogen in contributing to an increased microvascular constriction and permeability during diseases such as hypertension, stroke, or diabetes that are accompanied by an increased blood content of fibrinogen. Supported by NIH grant HL080394 to DL.

PPE4

ANGIOPOIETIN-1 AND VEGF ALTER ALBUMIN REFLECTION COEFFICIENT OF ISOLATED MOUSE GLOMERULI EX VIVO

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Glomerular reflection coefficient to albumin ($_{alb}$) represents integrity of the glomerular filtration barrier (GFB). describes the fraction of molecules retained by a membrane: =1 for molecules perfectly retained (e.g. >100kDa dextran); for albumin, <1. An albumin solution exerts a lower across the GFB than an iso-oncotic solution of dextran. The ratio of these effective is used to calculate $_{alb}$. We purified >100kDa dextran by dialysis, then created iso-oncotic dextran and BSA solutions. Glomeruli were sieved from C57bl6 mouse kidneys, aspirated onto a micropipette within a flow-controlled chamber, then perifused with BSA and examined under static conditions. Perifusate was then switched to dextran. Glomerular volume during each perifusate incubation period (dextran: V_{dex}; albumin: V_{alb}) was recorded on video, and calculated using ImageJ and Adobe Photoshop software. V_{dex}/V_{alb} describes $_{alb}$. Paired glomerular $_{alb}$ measurements were obtained before and after exposure to control solution, 200ng/ml Ang1, 40ng/ml VEGF, or both. Treatment with Ang1 caused a significant [p<0.05] percent increase in $_{alb}$ [0.71±0.24, n=15] compared with control [-0.30±0.34, n=15], as did VEGF [2.98±1.05, n=7] and Ang1+VEGF [2.26±0.75, n=7]. The increase in $_{alb}$ indicates a reduction in the macromolecular permeability of the GFB after treatment with both Ang1 and VEGF. Supported by Kidney Research UK and the British Heart Foundation

PPE5

DIABETIC MILIEU MODULATES GLOMERULAR ENDOTHELIAL GLYCOCALYX

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The podocytes and the glomerular endothelial cells (GEnC) are the key components of the glomerular filtration barrier (GFB). We have recently shown that GEnC are covered by a thick layer of glycoproteins called glycocalyx, which constitutes a barrier to the permeability of albumin. Dysfunction of the GFB leads to proteinuria, which is a hallmark of onset of diabetic kidney disease.

In this study we aim to test the effects of high glucose on the structural components of GEnC glycocalyx and examine its functional relevance.

We used conditionally immortalised human GEnC developed in our laboratory. Cells were cultured in normal or high glucose or osmotic control media. Expression of proteoglycan core proteins was analysed by western blotting. Biosynthesis of GAG chains was quantified by radiolabelling studies (3H glucosamine and S^{35}). mRNA expression of GAG biosynthetic enzymes was analysed by Taqman real-time PCR. Barrier properties of GEnC monolayers were studied by trans-endothelial electrical resistance (TEER) and passage of labeled albumin.

High glucose reduced expression of total and sulphated GAG chains by 55% and 62% respectively compared to controls (n=4, p<0.0001). There was however no significant change in the expression of proteoglycan core proteins. Functional studies showed no changes in the TEER, after high glucose (suggesting intact paracellular pathways) but a significant increase in the passage of albumin by 30% relative to controls (n=10, p<0.001).

In this study we show that high glucose reduces the expression and biosynthesis of GAG chains of glycocalyx without any alterations in their core proteins. Further, loss of glomerular endothelial glycocalyx also increases passage of albumin across GEnC monolayers. These results support the role of GEnC glycocalyx in the pathophysiology of proteinuria in diabetes. Funding: Kidney Research UK

PPE6

MECHANOSENSITIVE HYALURONAN (HA) SECRETION; STIMULUS-RESPONSE CURVES AND PHOSPHOLIPASE C-DEPENDENCE *IN VIVO*.

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HA influences interstitial and glycocalyx permeability, lubricates joints and conserves synovial fluid. Intra-articular (i.a.) HA injections and exercise ameliorate osteoarthritis. We therefore investigated how movement affects synovial HA secretion rate, *qHA.* Endogenous HA was washed out from cannulated knee-joint cavities in anaesthetised rabbits. Joints were cycled passively at 0-1.5Hz for 0-9min in every 15min for 5h. Newly secreted HA was harvested at 5h for HPLC. Movement at 0.5Hz-20% duration almost doubled *qHA* (p<0.0001, paired *t* test, n=35). Stimulus-response relations were curvilinear for movement frequency and duration. Since a Ca^{2+}-PKC–MEK-ERK pathway mediates stretch-stimulated *qHA* in culture (Momberger et al 2006 Matrix Biol 25 306), its functionality was tested *in vivo.* Ca^{2+} ionophore ionomycin doubled *qHA* in static joints (p=0.02, n=5 pairs), as did PKC activator PMA. In static joints PKC inhibitor bisindolylmaleimide (BIM) inhibited PMA-stimulated secretion (p<0.02, n=10,16, *t* test), as did MEK-ERK inhibitors U0126 and PD98059 (p 0.001, n=5 pairs). Phospholipase C (PLC) inhibitor U73122 almost fully blocked stimulation by movement (p=<0.001, n=10 pairs) but not static *qHA*. BIM, U0126 and PD98059, however, did not inhibit movement-stimulated *qHA* (p=0.32-0.96, n=5-11 pairs). Therefore,

HA secretion is a graded function of mechanical stimulation; and PLC (but not PKC-MEK-ERK) may be an obligatory transduction step *in vivo.*

PPE7

MEASUREMENTS OF WHOLE BODY GLYCOCALYX VOLUME: SOME QUESTIONS

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Measurements of the luminal endothelial glycocalyx (EGL) volume have great potential in clinical investigation. Recent estimates (Nieuwdorp et al. *Diabetes* 2006, 55: 480-486) are based on differences between the initial volumes of distribution of Dextran 40 (D40), which enters the EGL, and labelled red cells, which are excluded. While this seems reasonable, the methods are described briefly, raising questions that need to be answered. First, does D40 concentration in glycocalyx water equal that in plasma water at equilibrium? Initial volume of distribution equals volume of water in which tracer is dissolved only if tracer equilibrates to the same concentration throughout the system. Second, commercial Rheomacrodex (a mixture of different sized dextran molecules with different vascular permeabilities) is used as D40 in these studies. Does not this compromise the underlying assumption that initial volumes of distribution can be calculated from plasma clearance only when clearance is the same before and after the first plasma sample? Finally, why were albumin or fibrinogen not used as plasma markers instead of red cells since these molecules are initially excluded from the EGL and they are less affected by rheological factors? The poster will discuss possible answers.

Wednesday, August 27th
Inflammation and leukocyte function
Poster Session – PIN

PIN1
DIFFERENTIAL EFFECT OF SOLUBLE FIBRINOGEN AS A NEUTROPHIL ACTIVATOR
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Fibrinogen is an acute phase protein that participates in the inflammatory response. It is a ligand for several leukocyte adhesion molecules, most notably the integrin CD11b/CD18, and is reported to play a role in leukocyte signaling with generation of second messengers, production of oxygen free radicals and cell adhesion. The objective of this study was to measure how different parameters of neutrophil activation are modified by fibrinogen. Neutrophils from healthy donors were isolated from peripheral venous blood and incubated (1,0x 10^6 cell/mL) with fibrinogen (0-400 mg/dL) for 15 minutes. Neutrophil activation was evaluated using two different flow cytometry methods: (i) Loading with the fluorescent probe dihydrorhodamine 123 (1 microM) to detect oxygen free radical production; (ii) Staining for the surface marker CD11b (integrin M) both in the non-activated and activated conformations. Our results show that fibrinogen leads to an increase in neutrophil free radical production. However, the membrane expression and activation of the integrin M is not modified in the presence of fibrinogen. The mechanisms involved in this differential effect of fibrinogen as a neutrophil activator are not yet understood and we will address this question in further studies. *(Supported by Fundação para a Ciência e Tecnologia: PTDC/SAU-OSM/73449/2006).*

PIN2
INFLUENCE OF ECOLOGICAL FACTORS ON THE USE OF BLOOD LYMPHOCYTES MEMBRANE RESERVE IN THE IN VITRO EXPERIMENTS
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The rheological lymphocytes' characteristics and primarily their deformation ability are referred to a number of the major factors determining blood flow in the microvessels. The use of the membrane reserve is one of the components of blood cells' deformation changes. The data we got earlier in the in vivo experiments indicate that the exogenous hyperthermia (+38˚C, 2h) and prolonged usage of water with high content of calcium (66 mg/L) facilitate the use of membrane reserve by blood leucocytes. The purpose of the concluded research was to study the changes of the usage of plasmalemma's reserve by blood lymphocytes of rats' in the in vitro experiments in different conditions. The cells' incubation in the solutions of NaCl (0.2 per cent) for 60 sec. served as a functional probe. The changes of the lymphocytes' size were measured in comparison with that in the isotonic solution. The results of the investigation have shown that by the heightened temperature in comparison with room temperature the usage of plasmalemmas' reserve is facilitated. However, the extension of cells' sizes in hypotonic medium was more evident at temperature +37˚C than at +42˚C. Similar results were obtained by the lymphocytes' incubation in calcium solutions: small concentrations (2.23 mmol/L) stimulate the use of the membrane reserve by the leucocytes while heightened (2.52 mmol/L) – block it. The influence of constant magnetic field (0.28 mTl) also embarrasses the usage of the plasmalemmas' reserve. Thus it was determined that the positive influence of the studied factors on the usage of the membrane reserve by lymphocytes was evident by their less intensity in the in vitro experiments in comparison with that at the organism level.

PIN3
THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EXPERIMENTAL GINGIVITIS
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OBJECTIVES: The aim of the present study was to examine the potential role of type II VEGF receptor (VEGFR-2) in the process of experimental gingivitis.
METHODS: Gingivitis was provoked in anaesthetized male Wistar rats by placing ligature around and between the lower incisors next to the gingiva and building light-curing resin composite material upon it in to facilitate plaque retention. Seven days later the VEGFR-2 antagonist ZM323881 (10 microl., 20 microg./ml) was applied to the gingiva next to the lower incisors. Selected venules of the gingiva were observed by vital microscopy combined with digital photography.
RESULTS: There was a significant vasodilation of venules in experimental gingivitis as compared to the control group (diameter: 1013 ± 157 microm. and 407 ± 51 microm., respectively). After the local application of ZM323881 there was a significant vasoconstriction as compared to the baseline diameter of the venules of the inflamed gingiva at 15., 30. and 60.

minutes, (84.81 ± 6.01 %, 81.81 ± 6.44 %, 82.47 ± 4.77 %, respectively). There was no change of venule diameter in the control group.
CONCLUSION: Our findings suggest, that VEGF may have an important role in the venodilation of the gingiva in experimental gingivitis. This study was supported by grants OTKA T042584 and T049708.

PIN4

COMPARATIVE STUDY OF PHAGOCYTE FUNCTION IN CRITICALLY ILL PATIENTS: RESPIRATORY BURST AND ADHESION MOLECULE EXPRESSION

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Surgical trauma, polytrauma, burn injury may induce systemic inflammatory response syndrome. Independently from the primary cause of SIRS, leukocyte activation and leukocyte-endothelial cell interactions are involved in the microcirculatory changes. The purpose of this study was to follow the time course of leukocyte activation in patients with burn injury, polytrauma and severe sepsis. Blood samples were collected from 26 burned, 10 septic, and 6 polytraumatized patients at the time of ICU admission, and 5 consecutive days thereafter. Production of reactive oxygen species (ROS) in whole blood was measured by chemiluminometry. Adhesion molecule expression was determined by flow cytometry. White blood cell count decreased in burned and polytraumatized patients during treatment, while it remained at high level in septic ones. Marked granulocytosis and lymphocytopenia was observed in all groups. ROS production was persistently high in septic patients, however in burned and polytraumatized ones a dramatic increase appeared only from the 3rd day of trauma. Adhesion molecule expression increased on granulocytes, but it was permanently below the control on monocytes. Our results showed trauma specific alterations in oxidative burst and adhesion molecule expression in critically ill patients reflecting the pathologic leukocyte-endothelial cell interactions and microcirculatory disorders. Supported by OTKA T060227 grant.

PIN5

ANTI-INFLAMMATORY EFFECTS OF ANTIBIOTICS WITHIN THE INTESTINAL MICROCIRCULATION IN EXPERIMENTAL SEPSIS

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Introduction: Although the benefit of antibiotic therapy in infectious inflammatory diseases remains unquestioned, little is known about the effects of these antibiotics on the inflamed microcirculation independent of their antimicrobial property. Aim of this study was to evaluate acute effects related to antibiotics administration upon the intestinal microcirculation, which plays a crucial role in the pathogenesis of sepsis and subsequent multi-organ failure.

Methods: Experimental sepsis was induced in 80 Lewis rats using the colon ascendens stent peritonitis model. Seven frequently used antibiotics were included in the study (10 mg/kg metronidazol - MET; 20 mg/kg imipenem/cilastatin – IMI; 25 mg/kg tobramycin – TOB; 70 mg/kg vancomycin – VAN; 5 mg/kg erythromycin – ERY; 25 mg/kg linezolid – LIN; 5 mg/kg tigecyclin – TIG). The antibiotics were administered as single i.v. bolus following 15 hours of observation time. Intestinal functional capillary density and leukocyte-endothelial interactions were evaluated using intravital microscopy one hour following antibiotic treatment. Additional experiments were performed in an abacterial setting with comparable microcirculatory disturbances (2 hours endotoxemia; n=80).

Results: Acute IMI or TOB administration, respectively, did not affect the intestinal microcirculation. VAN treatment aggravated leukocyte rolling behavior during experimental sepsis. MET, ERY, LIN and TIG administration significantly reduced leukocyte activation and improved functional capillary density within the intestinal microcirculation both in experimental sepsis and endotoxemia.

Conclusions: The data suggest that some of the study antibiotics exert anti-inflammatory effects in addition to their antibacterial action. In conjunction with microbial sensitivity tests, potential beneficial effects of the antibiotics within the intestinal microcirculation should play a role in selecting the appropriate antibiotic therapy for patients with sepsis.

PIN6

A SECRETED COLLAGEN- AND FIBRONECTIN-BINDING STREPTOCOCCAL PROTEIN MODULATES CELL-MEDIATED COLLAGEN GEL CONTRACTION AND INTERSTITIAL FLUID PRESSURE

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Streptococcus equi subspecies *equi* and subspecies *zooepidemicus* are pathogenic horse bacteria. Subspecies *zooepidemicus* is considered as an opportunistic pathogen while subspecies *equi* causes the severe respiratory disease strangles. Both subspecies express structurally similar fibronectin-binding proteins, but whereas the *zooepidemicus* protein FNZ is cell wall bound, a frame shift mutation in the gene coding for the corresponding protein in *equi* result in a secreted

protein denoted FNE. We have investigated the effect of FNE in *in vitro* cell-mediated collagen gel contraction and *in vivo* measurement of the interstitial fluid pressure (P_{IF}). FNE stimulated collagen gel contraction mediated by murine C2C12 cells in an integrin □V□3-dependent manner. The process was dependent on fibronectin. FNE was able to bind both collagen and fibronectin and increased binding between immobilized collagen and soluble fibronectin by creating a molecular bridge between the two. *In vivo*, FNE normalized a P_{IF} lowered by degranulation of mast cells and our data suggests that it did so by stimulating the connective tissue cell contraction. We hypothesized that the ability of subspecies *equi* to compact the connective tissue makes it less accessible to the innate immunity and could be one explanation for the higher virulence of subspecies *equi* compared to subspecies *zooepidemicus*.

PIN7

ALTERED MONOCYTE CD44 EXPRESSION IN PERIPHERAL VASCULAR DISEASE IS CORRECTED BY FISH OIL SUPPLEMENTATION.

J Madden, CP Shearman, RL Dunn, ND Dastur, RM Tan, GB Nash, GE Rainger, A Brunner, PC Calder, RF Grimble

CD44 and its splice variants can be expressed on all leukocytes. They confer adhesive properties and enhance cellular recruitment across the endothelium during inflammation. CD44 expression is increased in inflammatory disease conditions such as atherosclerosis and CD44 variant 3 (CD44v3) expression may be associated with inflammation. We have examined CD44 and CD44v3 expression on peripheral blood monocytes before and after culture, with and without LPS stimulation. Monocytes were from patients with atherosclerotic peripheral arterial disease (PAD) or non-atherosclerotic controls. Both groups received dietary supplementation with fish oils.

Blood monocytes from PAD patients had increased CD44 expression compared to controls (Median Intensity Fluorescence (MIF) 480 $\pm$ 278 vs 336 $\pm$ 251 (mean SD); $p<0.001$). Following 12 weeks dietary supplementation with fish oil blood monocyte CD44 expression was reduced in PAD patients (MIF 480 $\pm$ 278 vs 427 $\pm$ 262; $p=0.05$) but not in controls (336 $\pm$ 251 vs 355 $\pm$ 280; ns). Isolated monocytes from both PAD and control subjects exhibited increased CD44 expression following 20 hour culture (953 $\pm$ 391 vs 2925 $\pm$ 1033 ($p<0.001$) and 875 $\pm$ 463 vs 2729 $\pm$ 1232 ($p<0.001$), respectively). However, following fish oil supplementation the culture-induced increase in CD44 expression was reduced (2925 $\pm$ 1033 vs. 2512 $\pm$ 882 ($p<0.001$) and 2729 $\pm$ 1232 vs 2364 $\pm$ 943 ($p<0.02$), (MIF before and after fish oil supplementation for PAD and control, respectively). CD44v3 expression was found to be lower in monocytes cultured from PAD patients compared to controls (0.15 $\pm$ 0.15 vs 0.22 $\pm$ 0.14 OD; $p<0.02$). This was increased in the PAD group following fish oil supplementation (0.15 $\pm$ 0.14 to 0.27 $\pm$ 0.23 ($p<0.001$). We have shown that the monocyte adhesion molecule CD44 expression is altered in vascular disease and that fish oil supplementation in vivo can alter both total CD44 and CD44v3 expression on monocytes.

PIN8

SPLENIC LYMPH FLOW AND LOCAL CYTOKINE PRODUCTION IN LIPOPOLYSACCHARIDE-INDUCED ACUTE INFLAMMATION IN THE RAT

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Insight into the properties of interstitial fluid representing the spleen microenvironment is important for understanding both the normal function of the spleen and its pathophysiology. Generally, lymph may be considered to be representative for interstitial fluid. Our aim was to study the effect on the spleen microenvironment of a systemic inflammation induced by lipopolysaccharide (LPS). Lymph was isolated by spleen lymph vessel cannulation. Spleen lymph was characterized by HPLC and showed a protein pattern similar to that in plasma in control rats as well as after LPS-treatment. Lymph flow increased significantly after LPS injection (3.0 mg/kg), whereas the colloid osmotic pressure and protein concentration in lymph and plasma fell significantly in LPS - treated rats, suggesting a low sieving of plasma protein in spleen vessels. LPS treatment resulted in a high local production of several proinflammatory cytokines (IL-2, IL-6, TNF-□) and the anti-inflammatory cytokine IL10. Flow cytometry analysis showed significantly increased T and B cell fluxes and thus mobilization to the general circulation after LPS injection. We conclude that access to the spleen microenvironment via lymph provides significant information on the role of the spleen in systemic inflammation.

PIN9

IMPACT OF BLOOD SHEAR-THINNING ON LEUKOCYTE RECRUITMENT IN WISTAR RATS

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Here we report data on leukocyte trafficking through venules of cremaster muscle of Wistar rats aiming at understanding the influence of blood rheology on the recruitment processes.

With *in vivo* intravital microscopy experiments, real time dynamics were recorded to monitor leukocyte trajectories and measure velocities and forces exerted on them.

Initial experiments revealed that Carreau-Yasuda model describes well a shear-thinning behaviour of blood viscosity within the measured shear rate range. A novel lattice Boltzmann shear-thinning blood flow solver was used to quantify localized hemodynamics on the surface of moving, individual and cluster, leukocytes and on the endothelial wall.
From 3D computer simulations for individual leukocytes, we observed that moving leukocytes strongly disturb the shear stress on the endothelial wall at large distances. One region of maximum and two of minimum shear stress are formed on the surface of each cell. Moreover, trapping vortices and stagnant regions favour leukocyte recruitment towards the wall.
Clusters of recruited leukocytes describe the same dynamics observed for single leukocytes, increasing the endothelial wall shear stress as the clusters move and with the formation of vortices and stagnation points. These computed simulations suggest that the shear-thinning behaviour of blood favour leukocyte recruitment, namely the rolling process.

PIN10

INCREASED SERUM VEGF AND GASTRIC MUCOSAL MACROMOLECULAR LEAKAGE IN HELICOBACTER PYLORI-INFECTED RATS

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Helicobacter pylori (*H. pylori*) infection causes chronic gastritis. *H. pylori* can activate many inflammatory mediators and gastric microcirculatory disturbance. This study aimed to determine *H. pylori* infection could induce serum vascular endothelial growth factor (VEGF) and gastric mucosal macromolecular leakage (MML) in rats. Ten male Sprague-Dawley rats were divided into control and *H. pylori*-infected groups. Rats were inoculated by gavage with normal saline or *H. pylori* suspension (5×10^8 to 5×10^{10} CFU/ml) twice daily at an interval of four hours for three consecutive days. After two weeks, the MML was examined by intravital fluorescence microscopy. Then, the stomach was removed for histological examination and blood sample was collected for serum VEGF study. Histological examination showed neutrophil infiltration in laminar propria of *H. pylori*-infected rats. *H. pylori* infection significantly increased serum VEGF ($p<0.000$) as well as the MML ($p<0.010$) compared with control group. The averages of VEGF concentrations were 228.57+/-40.41 and 619.43+/-145.68 pg/ml, and the averages of MML were 10.69+/-1.43 and 15.41+/-2.83% in control and *H. pylori*-infected groups, respectively. Therefore, *H. pylori* infection increased serum VEGF, gastric mucosal MML, and neutrophil infiltration. These results suggested that MML in *H. pylori*-infected rats may be mediated by VEGF expression. This study was supported by Thailand Research fund (code RMU 4980032) and Graduate Thesis Grant, Graduted School, Chulalongkorn University (2005).

PIN11

THE HUMAN INTERFERON ALPHA (IFNα) EFFECTS ON CYTOKINE PRODUCTION AND CELL ADHESION MOLECULES (CAM) EXPRESSION BY HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) INFECTED HUVEC.

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The majority of human population is infected with HSV-1 causing latent recurrent infection. HSV-1 persists in neurons and is also found in human vascular endothelial cells. Pro-inflammatory modification of endothelium induced by HSV-1 is believed to be a step toward atherogenesis. The efficacy of IFNα therapy during herpetic disease relapses is limited. The purpose of this study was to evaluate the effect of IFNα2b on HSV-1 replication in cultured HUVEC and on the pro-inflammatory modification of HSV-1 infected HUVEC. Experimental design implied cell treatment with varying doses of IFNα2b prior to, concomitantly, and after the infection of cells at various multiplicity of infection (MOI). IFNα2b was found to stimulate the production of IL-1β, IL-8, and TNFα by HUVEC infected with HSV-1; the effect was dependent on MOI, IFNα2b concentration, and experimental design. IFNα2b influenced on CAM shedding from HUVEC infected with HSV-1. The levels of soluble P-, E-selectin, ICAM-1, and VE-cadherin were found to be increased in the culture medium of HSV-1 infected HUVEC treated with IFNα2b. It can be concluded, therefore, that IFNα2b effect on HUVEC infected with HSV-1 is far from being unambiguous; the well-known anti-inflammatory action of interferon may also involve activation of inflammatory process. The study was supported by Grant ISTC 3505

PIN12

THE MAMMALIAN ACTIN BINDING PROTEIN 1 IS REQUIRED FOR FIRM ARREST AND INTRALUMINAL CRAWLING OF NEUTROPHILS

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The mammalian actin-binding protein 1 (mAbp1, HIP-55) is phosphorylated by Syk, a tyrosine kinase that is important for neutrophil (PMN) recruitment during acute inflammation. Here we studied the role of mAbp1 for beta 2 integrand-mediated PMN adhesion and migration using mAbp1 deficient mice and Europhile-like differentiated HL-60 (dHL-60) cells. Upon adhesion on immobilized fibrinogen, mAbp1 was translocated to the leading edge of polarized dHL-60 cells and murine PMN. This process was impaired in dHL-60 cells upon inhibition of Syk using piceatannol indicating a role of Syk for the translocation of mAbp1. Under static conditions, mAbp1 was not required for adhesion and migration. In contrast, mAbp1 was essential for PMN adhesion in the inflamed murine cremaster model and for migration under physiologic shear stress conditions. In comparison to control cells, the expression of mutated mAbp1 (mAbp1-EGFP Y334F/Y344F) in dHL-60 cells

led to an impaired translocation of the Wiskott-Aldrich syndrome protein (WASP) to the leading edge. Thus, mAbp1 seems to be required for the processes of intraluminal firm arrest and crawling under shear stress, probably via the control of the subcellular localization of WASP. Taken together, we identified mAbp1 as a novel component in the molecular mechanism underlying PMN recruitment in vivo. (Supported by DFG Wa 1048/2-3)

PIN13

INFLUENCE OF CERULOPLASMIN ON PLATELET-LEUKOCYTE AND ERYTHROCYTE-LEUKOCYTE INTERACTIONS IN BLOOD DURING THE EARLY STAGES OF EXPERIMENTAL ABDOMINAL SEPSIS DEVELOPMENT

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Endothelial activation and changes in cell rheology that occur during sepsis play an important role in impairing microvascular blood flow. Ceruloplasmin (CP), a copper-containing acute phase protein, is available as pharmaceutical preparation and has the ability to stabilize cell membranes, inhibit lipoperoxidation. The goal of this study was to investigate influence of CP on platelet-leukocyte (PLI) and erythrocyte-leukocyte (ELI) interactions during the early stages of abdominal sepsis development. The study was carried out on 60 male Wistar rats (275-325 grams weight). Abdominal sepsis was induced by cecal ligation and puncture (CLP). Development of acute peritonitis was confirmed by pathohistological methods. CP ("Microgen", Russia) was infused intravenously in single dose 30 mg/kg. Animals sacrificed at 3, 6 and 12 hours after CLP. Cell-cell interactions were evaluated by the original method, based on microscopic count of cell aggregates. Administration of ceruloplasmin leads to normalization of ELI ($p<0.05$). PLI were reduced significantly, but did not reach normal figures ($p>0.05$). In summary, CP normalizes cell-cell aggregation in blood during the early stages of experimental abdominal sepsis development. Probably, this is due to stabilization of cell membranes by antioxidant action of ceruloplasmin. These results enhancing current knowledge about ceruloplasmin as acute phase protein.

PIN14

FROM ROLLING TO ARREST: LEUKOCYTE RECRUITMENT DURING FETAL DEVELOPMENT

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Leukocyte recruitment describes the ability of leukocytes to leave the intravascular compartment and transmigrate into tissue. Leukocyte recruitment follows a cascade of events, which is well-defined in adult organisms. However, little is known about the recruitment of leukocytes in fetal tissue. Using a recently developed model of intravital microscopy in the fetal mouse, we observed interactions between neutrophils and the endothelial lining within unstimulated blood vessels of the yolk sac (YS). To specifically investigate neutrophil rolling and adhesion in YS vessels, we used LysEGFP mice, which specifically express GFP in neutrophils (Faust et al., Blood 2000). Interestingly, our results demonstrate that rolling of GFP-neutrophils within YS vessels is chronologically acquired during gestation, beginning at around embryonic day 14 (E14) and reaching a plateau at E17-E18. Similarly, neutrophil adhesion in unstimulated yolk sac vessels was rarely observed at or before E14 fetuses but gradually increased thereafter. In contrast, hemodynamic parameters in the YS remained comparable between E14 and E18. Our findings extend the multistep model of leukocyte adhesion to the mid- and late-term fetus and indicate that neutrophils reach sites of inflammation by obeying similar traffic rules as leukocytes in postnatal life.

PIN15

THE NOCICEPTIN ANTAGONIST UFP101 REDUCES MICROVASCULAR INFLAMMATION TO LIPOPOLYSACCAHRIDE *IN VIVO*

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Nociceptin/orphanin FQ (N/OFQ) is an 'opioid-like' peptide and an endogenous ligand for the NOP receptor (also known as ORL1). Within the microcirculation, it causes dilation and inflammation (Brookes *et al.* Am J Physiol Heart Circ Physiol 2007;293:H2977-85). As sepsis involves generalised inflammation of blood vessels, we hypothesised that the function of microvascular NOP receptors would be altered by lipopolysaccharide (LPS). Male Wistar rats (n=24) were anaesthetised (thiopental: 40mg.kg^{-1} bolus induction, 1-100mg.kg^{-1}hr^{-1} maintenance) to measure macromolecular leak and leukocyte activation (rolling and adhesion) within mesenteric venules (<40microns) using fluorescent intravital microscopy (FITC-BSA; 0.2ml.100g^{-1}, i.v). LPS, into the tail vein at 24 hrs (1mg.kg^{-1}) and 1 hr (0.5mg.mg^{-1}) prior to experimentation, caused an increase in leukocyte rolling (control: 2.8 $\pm$1.2 LPS: 4.2 +/-2.3 per min) and leak (control: 26.3 +/-8.0, LPS: 132.4 +/-16.5%, $p<0.05$), both reduced by the specific NOP antagonist UFP101 (150nmol.kg^{-1}, i.v) (Calo G *et al*. CNS Drug Rev. 2005;11:97-112) (rolling: 0.6 $\pm$0.4, leak: 39.1 +/-14.4, $p<0.05$). Antagonism of NOP receptors appears to reduce microvascular inflammation (leukocyte rolling and macromolecular leak) in a LPS model of sepsis. Further work is required to elucidate the mechanism of this. This work was funded by a *British Journal of Anaesthesia* project grant. EN Stedman was the recipient of an *Anaesthetic Research Society* summer studentship.

PIN16

THE ROLE OF LYMPHOTOXIN IN REGULATING TRANSCAPILLARY FLUID BALANCE IN MOUSE SKIN

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Lymphotoxin (LT) α and β are cytokines belonging to the tumor necrosis factor superfamily that mediate inflammation and lymphoid organ development. LTα-/- mice lack all peripheral lymph nodes and Peyer's patches, while LTβ-/- mice retain only mucosal, cervical and sacral lymph nodes. LTα-/- and LTβ-/- mice were evaluated for a phenotype affecting extracellular fluid balance in the skin. Interstitial fluid pressure (IFP), colloid osmotic pressure (COP) in plasma and interstitium and extracellular fluid volume (EFV) were determined in both steady state and following intravenous overhydration with Ringer-acetate using micropipettes connected to a servo-null system, a colloid osmometer and ^{51}Cr-EDTA, an extracellular tracer respectively. The LTα-/- animals showed a significantly higher IFP in the steady state compared to the LTβ-/- and wild type (WT) mice. The IFP increased following overhydration in all three groups with no significant difference between them. In the overhydrated LTα-/- mice, the COP in interstitial fluid was higher, and the COP gradient over the capillaries was lower when compared to WT animals. The EFV increased significantly in both LTβ-/- and WT mice following overhydration, in contrast to the LTα-/- mice. These data demonstrate a role for LTα in the regulation of transcapillary fluid balance in mouse skin. Supported by Western Norway Regional Health Authority (OSS), NIH grants RO1 DK57731 (NHR), CA 16885 (NHR), and a Lymphatic Research Foundation Postdoctoral Fellowship Award (RHM).

PIN17

A FUNDAMENTAL ROLE OF THE MAMMALIAN ACTIN BINDING PROTEIN 1 FOR CD11/CD18-MEDIATED PHAGOCYTOSIS OF COMPLEMENT-OPSONIZED BACTERIA IN NEUTROPHILS

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The mammalian actin-binding protein 1 (mAbp1, SH3P7, HIP-55) is a substrate of the non-receptor tyrosine kinase Syk which is known to be critically involved in complement-mediated phagocytosis. Here we studied the role of mAbp1 for the engulfment of iC3b-opsonized E. coli in mAbp1 deficient neutrophils (PMN) and in neutrophil-like differentiated HL-60 (dHL-60) cells upon downregulation of mAbp1 using RNA interference technique (siAbp1). The genetic absence or the downregulation of mAbp1 significantly impaired CD11b/CD18-mediated phagocytosis in PMN and in dHL-60-siAbp1 cells. However, the expression of the complement receptor CD11b/CD18 or the PMN differentiation marker GR-1 was unaffected in mAbp1 deficient PMN compared to control cells. Impaired phagocytosis of E. coli particles was also observed in murine Syk deficient PMN. Furthermore, the pharmacological inhibition of Syk in dHL-60 cells or its genetic absence in murine PMN led to an impaired translocation of mAbp1 to the site of particle binding indicating that Syk-mediated recruitment of mAbp1 was required for efficient phagocytosis. The fundamental role of mAbp1 in phagocytosis was confirmed by live cell imaging using dHL-60 cells expressing EGFP-tagged mAbp1. Thus, we identified the Syk substrate mAbp1 as a novel regulator of complement-mediated phagocytosis in PMN. This study was supported by DFG Wa1048/2-3.

PIN18

DELAYED NEUTROPHIL APOPTOSIS IN CHRONIC POLYARTHRITIS PATIENTS WITH LESS THAN 6 WEEKS OF DURATION

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Early polyarthritis (PA) can evolve into different chronic diseases or they can spontaneous enter into remission. Neutrophils are clearly relevant in the early phase of a polyarthritis and their abnormal survival could contribute for the perpetuation of an initial inflammatory process. In this study we analysed the apoptosis of circulating neutrophils in untreated and treated very early PA (less than 6 weeks) and compare the differential susceptibility to apoptosis in 3 groups of patients, according to their latter clinical evolution: rheumatoid arthritis (RA), other forms of chronic arthritis (CA) and self limited polyarthritis (SLPA). Apoptosis was analysed by the DNA content using propidium iodide. RA patients without any treatment or treated with corticosteroids showed a delay of neutrophil apoptosis at 6 h and 22h in culture compared with neutrophils from healthy controls. Delayed PMN apoptosis was restored to control levels after treatment with methotrexate. The delay of apoptosis was also observed in other CA patients. In contrast, neutrophil apoptosis from SLPA patients did not show any differences compared with the controls. These results suggest that prolonged neutrophil survival might trigger the perpetuation of a chronic form of arthritis and highlights the importance of early RA treatment with methotrexate.

PIN19

ENDOTHELIAL ICAM-1, VCAM-1 AND E-SELECTIN EXPRESSION IS DOWNREGULATED BY HYPERBARIC OXYGEN IN AN IN VITRO CHRONIC WOUND MODEL

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Hyperbaric oxygen (HBO) therapy [the intermittent inhalation of 100% oxygen, at a pressure greater than 1 atmosphere absolute (ATA)] is used to treat chronic wounds. These wounds experience excessive neutrophil accumulation that contributes to the persistent inflammation observed. Neutrophil recruitment is regulated by endothelial cell (EC) adhesion molecules including ICAM-1, VCAM-1 and E-selectin. This work examines whether HBO reduces EC adhesion molecule expression. This could aid wound healing by reducing neutrophil accumulation. Human umbilical vein ECs were treated with 0.5µg/ml lipopolysaccharide and 1ng/ml tumour necrosis factor alpha at 2% oxygen (i.e. hypoxia), which simulates the chronic wound environment and induces adhesion molecule expression. After HBO treatment for 90 minutes at 2.4 ATA (patient treatment protocol) adhesion molecule expression or release was measured by ELISA at 0, 5, and 24 h post-treatment. At 24 h post-treatment, cells treated with HBO expressed only 53.4±12.8% (p=0.038, n=16) of ICAM-1 and 88.1±29.4% of VCAM-1 (p=0.017, n=18) compared with control cells (100%). HBO-treated cells also released 0.85±0.17ng/ml E-selectin compared with 0.66±0.18ng/ml in control cells (p=0.041, n=6). Protein assays confirmed these results were not due to cell loss. In conclusion, HBO treatment significantly reduced ICAM-1 and VCAM-1 expression and increased E-selectin release in human ECs. Funded by the Diving Disease Research Centre, Plymouth, UK and the Peninsula Medical School, Universities of Exeter and Plymouth, UK.

PIN20

BLOOD FLOW-DEPENDENT ARTERIAL REMODELING IS FACILITATED BY INFLAMMATION BUT DIRECTED BY VASCULAR TONE

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Aim: Altered blood flow affects vascular tone, attracts inflammatory cells, and leads to microvascular remodeling. We tested the hypothesis that inflammation facilitates the remodeling response, but that vascular tone determines its direction.
Methods: Blood flow was altered in arcading mesenteric arteries of wild type mice by a surgical ligation, creating low flow, high flow and normal flow vessels. In vivo microscopy was used to determine changes in vascular tone. Structural remodeling was studied after two days, with or without macrophage depletion. In order to characterize the inflammatory response, immunostaining, confocal microscopy and real-time PCR were used. To address the role of vascular tone in remodeling, inflamed and control vessels were exposed to vasodilator treatment with amlodipine for 24 hours.
Results: Vessels exposed to high blood flow dilated, whereas low flow induced constriction. After one day, the number of adventitial macrophages increased in both high and low flow and the inflammatory markers showed a complex but remarkably similar increase in expression. Depletion of macrophages eliminated flow-induced remodeling. Manipulation of vascular tone reversed inward remodeling in response to low blood flow.
Conclusion: Altered blood flow triggers an inflammatory response that facilitates remodeling. Vascular tone is a crucial determinant of the direction of the remodeling response.
Supported by the Netherlands Heart Foundation (NHS 2001T038).

Wednesday, August 27th
Haemostasis and platelet function
Poster Session - PHP

PHP1

PROTEIN DISULFIDE ISOMERASE (PDI) MEDIATES TISSUE FACTOR (TF)-DEPENDENT FIBRIN FORMATION IN VIVO

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In intact blood vessels, Tissue factor (TF) and consequently intravascular fibrin generation is only minimally active, and the coagulation system is down-regulated. In contrast, TF is rapidly stimulated following endothelial disruption. However, the stimuli responsible for inducing TF activation are largely undefined. Here, we have identified a novel pathway for TF activation *in vitro* and contributes to intravascular coagulation *in vivo*. We show that the protein disulfide isomerase (PDI) induces disulfide formation of the Cys186/Cys209 pair of TF. *In vitro*, PDI-mediated disulfide formation led to profound activation of TF. *In vivo,* we found that PDI is not present in the intact vessel wall, but following endothelial denudation, injured smooth muscle cells and adherent/activated platelets are the major sources of abundantly expressed PDI at sites of vascular injury. Using intravital microscopy, following injury of carotid artery, we observed that PDI-blockade strongly reduced fibrin formation by approximately 45-60%. Likewise, i.v. infusion of PDI significantly enhanced TF-dependent fibrin formation. Together, this implies that PDI initiates coagulation *in vitro* and also during arterial thrombosis *in vivo*. Notably, PDI did not mediate platelet-activation. Correspondingly, PDI blockade also inhibited fibrin formation, when platelet adhesion was prevented by GPVI-blockade. These findings reveal for the first time *in vivo* that PDI is a major trigger of coagulation, which is exposed after vessel injury and converts TF into its functionally active form.

PHP2

CHRONIC VENOUS INSUFFICIENCY IS ASSOCIATED WITH ELEVATED LEVEL OF CIRCULATING MICROPARTICLES

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Objective - Microparticles (MPs) are small membrane vesicles released by several circulating and vascular cells upon activation or apoptosis. The purpose of this study was to assess the subpopulations of circulating endothelial (EMPs) and platelet microparticles (PMPs) in chronic venous insufficiency (CVI), and to disclose their contribution in mediating dysfunction of human peripheral venules.

Methods and Results - Human peripheral venules were explanted from patients with CVI, and from control subjects (C); concurrently, blood samples were collected and circulating MPs isolated. The techniques used were: flow cytometry, electron microscopy, fluorescence microscopy, and the myograph technique. The results showed that compared to controls, patients with CVI had: (i) a marked elevations of circulating EMPs and PMPs, (ii) structural modification of the venous wall consisting in activation of endothelial and smooth muscle cells, abundance of intermediary filaments and synthesis of hyperplasic-multilayered basal lamina, (iii) altered reactivity of the venous wall, as a consequence of EMPs and PMPs adherence; altered contractile response to noradrenaline, acetylcholine, 5-hydroxytryptamine, and KCl and an impeded endothelium relaxation to sodium nitroprusside.

Conclusions - The findings indicate that CVI is accompanied by an enhanced release of EMPs and PMPs that contribute to altered dysfunctional response of the venous wall. This study was supported by the Excellence Research Projects for Young Researchers (Grant no. 15121/2006-2008) financed by Ministry of Education and Research, Romania.

PHP3

COLLAGEN RECRUITS PLATELETS VIA GPIb-IX-V TO MUCOSAL VILLI FOLLOWING MURINE INTESTINAL ISCHAEMIA – REPERFUSION (IR) INJURY *IN VIVO*

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Intestinal mucosal villi are very susceptible to IR injury. Previous studies suggest platelets contribute to injury following recruitment by intact endothelium. However, IR injury can lead to endothelial denudation and subsequent collagen exposure. We hypothesise platelet-collagen interactions lead to platelet recruitment following I/R injury. Therefore, roles for platelet collagen glycoprotein receptors, GPVI and integrin $_{2\ 1}$, and the indirect collagen receptor GPIb-IX-V (via VWF), were determined intravitally. Intestinal IR injury was induced for 45 min in anaesthetised (ketamine/xylazine) C57BL/6 (WT) or FcR -chain$^{-/-}$ (lack GPVI) mice. Some WT mice were perfused with function blocking mAb's to $_{2\ 1}$ (SAM G4) or GPIb (Xia-B2). Donor and endogenous platelets were fluorescently labelled to quantitate singular and aggregated platelets respectively. Large microthrombi and diminished blood flow was observed in jejunal IR injured villous microvessels. In

contrast, single adherent platelets, as well as fewer/smaller aggregates and maintained blood flow were observed in the ileum. Platelet recruitment was not significantly different in FcR -chain$^{-/-}$ mice or those pre-treated with $_{2\ 1}$ mAb. In contrast, significantly smaller ($p<0.001$) and less fluorescently intense ($p<0.001$) microthrombi with improved blood flow was observed jejunally in animals pre-treated with GPIb mAb. Targeting GPIb-IX-V may prove beneficial in improving clinical morbidity associated with intestinal I/R injury. Funded by the British Heart Foundation

PHP4

CC CHEMOKINES CCL2/JE/MCP-1 AND CCL3/MIP-1 MEDIATE NEUTROPHIL RECRUITMENT VIA ENDOGENOUS GENERATION OF PLATELET-ACTIVATING-FACTOR

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Recent studies suggest that CC chemokines including CCL2/JE/MCP-1 and CCL3/MIP-1 are critically involved in the recruitment of neutrophils. However, the underlying mechanisms remain unclear. In the present study, leukocyte rolling, firm adherence, and transmigration were analyzed in the mouse cremaster muscle using *in vivo* microscopy. After 180 min of stimulation with CCL2/JE/MCP-1 and CCL3/MIP-1 , no significant differences were found in numbers of rolling leukocytes among experimental groups (n=6). In contrast, stimulation with CCL2/JE/MCP-1 and CCL3/MIP-1 induced a significant increase in the number of firmly adherent and transmigrated leukocytes (> 80% Gr-1^{+} neutrophils) as compared to controls. This increase was significantly diminished in mice receiving an inhibitor of RNA synthesis (actinomycin D) or a platelet-activating-factor (PAF) receptor antagonist (BN52021), respectively. Moreover, stimulation with CCL2/JE/MCP-1 and CCL3/MIP-1 caused a significant elevation in the leakage of FITC dextran as compared to controls. This elevation was almost completely abolished in mice treated with actinomycin D and BN52021, respectively. In conclusion, these *in vivo* data demonstrate that CCL2/JE/MCP-1- and CCL3/MIP-1 -elicited firm adherence and (subsequent) transmigration of neutrophils as well as microvascular permeability are dependent on protein synthesis and endogenous generation of proinflammatory mediators such as PAF. This study was supported by Deutsche Forschungsgemeinschaft (FOR440).

PHP5

THE ENDOTHELIAL GLYCOCALYX: MODERATOR OF PLATELET-VESSEL WALL INTERACTIONS IN ATHEROGENESIS?

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The endothelial glycocalyx (EG) is the luminal cover of endothelium, involved in modulation of blood cell adhesion. Early in atherogenesis, platelets interact with the endothelium of the carotid bifurcation. We evaluated the role of the EG in these interactions in control and ApoE-/- mice (7-30 weeks). Spontaneous interactions of fluorescently labeled platelets were visualized in vivo in the carotid artery (intravital microscopy). Afterwards, carotids were mounted in a vessel chamber and perfused with fluorescently labeled lectin to visualize the EG (two-photon microscopy).
Platelet-endothelium interactions were classified as short (+/-100 msec) or long (>20 sec). Whereas no changes in short interactions occurred, long interactions increased with age in the ApoE-/- bifurcation (from 32 platelets/mm^2 at 7-13 weeks to 56 at 25-30 weeks ($p<0.05$); 0-16 in controls). EG thickness increased with age in the control bifurcation from 4.2 microm (7-13 weeks) to 6.4 microm (25-30 weeks; $p<0.05$); in ApoE-/- mice it remained 4.1 microm. Pilot data suggest higher heterogeneity in EG thickness in ApoE-/- carotids. Further research is aimed at elucidating whether the reduced average thickness and suggested higher heterogeneity of the EG in lesion-prone arteries of ApoE-/- mice are causally related to the increased platelet-endothelium interactions in these vessels. Supported by NWO grant #902-16-276 and SenterNovem (BSIK 03033)

PHP6

ETHANOL EXTRACT FROM MRRUBIUM INCISUM INTERVENCE THE ABNORMAL HEMORRHEOLOGICAL IN ACUTE MICROCIRCULATORY DISTURBANCE RATS

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Male Wistar rats were divided into *Mrrubium Incisum* (MI) group, model group, control group. The acute microcirculatory disturbance (AMD) model was established by intravenously administrating Dextran 500 (10ml/kg.bw). 6min later, ethanol extract from *Mrrubium Incisum* (EEMI) was infused in MI group (1g/ml, 6g/kg), and EEMI was replaced by normal saline in other groups. After 40min, the hemorheology indexes, electrophoresis and erythrocytes deformability, platelet function were observed. Compared with control group, the whole blood, relative and plasma viscosity, Hct in model group and the ESR, electrophoresis time, platelet adhesive rate, disaggregation rate and time in model and MI group were increased significantly, the platelet aggregation rate in model group and the electrophoresis length, erythrocyte migration, cerebral blood flow, thrombosis length, wet and dry weight of thrombosis in model and MI group were lower significantly. However, the whole blood, relative and plasma viscosity, HCT, platelet adhesive rate, thrombosis dry weight in MI group were lower, the platelet aggregation rate and erythrocyte deformability were increased significantly than that of model group,

respectively. The results demonstrated EEMI could reduce the viscosity, platelet adhesive and thrombosis forming, and raise erythrocyte deformability of AMD rats. The work was supported by NSFC (30770845) and NSFHebei (C2008000503).

PMI1

TRANSCUTANEOUS PO_2 IMAGING DURING TOURNIQUET-INDUCED FOREARM ISCHEMIA USING PLANAR OPTICAL OXYGEN SENSORS

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Oxygen-dependent quenching of luminescence using transparent planar sensor foils was shown to overcome the limitations of the polarographic electrode technique in an animal model. This method was now transferred to a clinical setting to measure the transcutaneous pO_2 ($p_{tc}O_2$).
In six healthy subjects, a cuff on the upper arm was occluded up to 20 mm Hg above systolic pressure and released after eight minutes. $P_{tc}O_2$ was measured at the lower arm every 30 seconds prior to, during, and up to 20 minutes after cuff occlusion (40 °C applied skin temperature) by using luminescence lifetime imaging of platinum(II)-octaethyl-porphyrin immobilized in a polystyrene matrix. For validation, the polarographic Clark electrode technique was applied in close proximity, and measurements were conducted simultaneously. $P_{tc}O_2$ measurements prior to (70.8 19.1 mmHg vs. 66.2±7.7 mmHg) and at the end of ischemic (2.7 1.2 mmHg vs. 3.6±1.7 mmHg) and reperfusion phases (72.2±3.6 mmHg vs. 68.4±8.9 mmHg) did not differ significantly using the Clark electrode vs. luminescence lifetime imaging. At both the initial ischemic and the reperfusion phases the Clark electrode measured a faster decrease or increase respectively in $p_{tc}O_2$ because of the oxygen consumption occurring in this method. The presented method provides accurate and reproducible $p_{tc}O_2$ values under changing microcirculatory conditions. The lack of oxygen consumption during measurement allows both a more realistic estimation of $p_{tc}O_2$ than compared to the gold standard and the permanent use in regions with critical oxygen supply.

PMI2

A MULTIMODAL OPTICAL IMAGING OF BLOOD VESSELS

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We present a multimodality approach for optical imaging of blood vessels. This approach includes three imaging modalities. 1) Brightfield Spectral Imaging (BSI), 2) Fluorescent Imaging (FI) and 3) Dynamic Light Scattering Imaging (DLSI). BSI is utilizing a conventional light source where reflected light passes through band-bass filter in order to enhance contrast of hemoglobin. FI is utilizing a standard light source for fluorescent microscopy plus contrast material for fluorescent angiography. DLSI is utilizing a diffuse coherent light source where reflected laser interference pattern carry information about red blood cell motility and total blood perfusion. Imaging is performed with minimal changes of optical components between modes. A Mouse ear model and Dorsal Skin Fold Chamber (DSFC) preparation were used to demonstrate the new multimodality imaging approach potential. These three modalities expand the standard microscope capabilities and provide additional information about functionality of different blood vessels.

PMI3

THE MODERN TECHNOLOGY OF SMART OPTICAL CAPILLAROSCOPY WITH PROGRAMMED QUANTITATIVE ANALYSIS AND CLINICAL INTERPRETATION

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Investigation of the vascular system in the human organism has always attracted attention of physicians and biologists. But nowadays investigation of vascular system requires a new technical approach and a good knowledge in hemodynamics and in angioarchitectonics. The essential advantage of diagnostic non-invasive methods is in possibility of their harmless many-time use in order to receive structural and functional changes in dynamics in an area of injury and adjacent sections. And it becomes possible to apply functional loads and realization of pharmacological tests that is extremely important for selection of treating tactics. Substantiating the problem of a human organism's senescence A. Zalmanoff (1958) considered that we could always observe the progressing decrease of number of open capillaries**,** a part of them transformed into a state of shadows – non-functioning capillaries, for people at the age of 40-45. This progressing drying makes the anatomic-physiologic base of senescence. People of the elderly age have a velocity of blood flow that is reduced in one third because of relative atony of capillaries in these people, their partial occlusion causes increasing of resistance in the peripheric blood

circulation. In the very senescence capillaries become weaker, winding, their diameter diminishes up to closed dots. Blood flow is slowing down. As the capillary blood circulation makes the main function of the microcirculatory system – transcapillary exchange, that's metabolism between blood and tissues, condition of the microcirculation can be as an arbiter of welfare in the systemic hemodynamics and reflects the preserved arteriovenous balance in the regional vascular reservoirs. Therefore scientists and practitioners again pay attention to a state of the microcirculatory link. Renascence of the forgotten capillaroscopy is natural on the present stage of development of visualizing technologies in vivo. Words of Zalmanoff A.S. (that are 50 years old) are again actual: "Disturbances of the capillary physiology are so widespread and are so often observed that they must not be considered as the secondary phenomenon but on the contrary as the one of the main elements of organic impairments in a patient suffering from any illness". Advantages of smart capillaroscopy over other methods of microcirculation investigation are in visualization of the process, that significantly makes easy the doctors' comprehension of capillary pictures, enables to study more deeply and to analyze obtained data. Processing the obtained images the application of the mathematical modeling enables in more details to analyze picture of the microcirculation, to present it in the figure characteristics that can be an addition to a quantitative assessment of these virtual sections and reflect slight changes in the quantitative equivalent. Main advantages of the smart capillaroscopy are the following: simplicity in work, non-invasive, high quality of visualization both of capillaries and the velocity of circulating blood, possibility of archivation both of separate static images and video files for processing obtained images in order to increase the contrasts and obtain maximally useful information. The following facts are very important for medical staff, who uses the smart capillaroscopy: mistaken diagnosis is practically excluded, results of the disease development is highly predictable, qualitative and quantities assessment of efficiency of the performed therapy, possibility of obtaining unique microcirculatory changes for prognostication of sub- and decompensated states of patients. For the last 12 years scientific, clinical-diagnostic and treating practice on the basis of the Ukrainian Scientific Methodical Center of Ultrasound Medical Diagnosis "Istyna" gradually required restoration of the pathohemodynamic link in the investigation of the vascular system "heart-arteries-capillaries-veins-heart". Very this circumstance of uncompleted cycle of the vascular blood supply for the brain was the main factor in revival of the method that was undeservedly forgotten. The capillaroscopy really deserves the place in the continuous chain of investigations of the pathohemodynamic link in the vascular system: heart-arteries-capillaries-veins-heart. The capillary canal is the end segment that is more distant from the center and simultaneously according to hemodynamics it is the most sensitive link in the vascular system. Regular capillaroscopic investigation enables to perform diagnostics of microcirculatory disorders, non-invasively to control efficiency of the therapy almost for every patient and also during prophylactic exanimations to control a state of railway men, pilots, astronauts etc, who are connected with a risk due to their professions.

PMI4

IN VIVO IMAGING OF MOUSE ELASTIC ARTERIES USING TWO PHOTON LASER SCANNING MICROSCOPY: A FEASIBILITY STUDY

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In vivo imaging of elastic arteries using two-photon laser scanning microscopy (TPLSM) results in images that suffer from motional artifacts within each image and between subsequent images. We applied TPLSM triggered on cardiac and respiratory cycle, and accelerated image acquisition to reduce the impact of motion between subsequent images and within each image.

Carotid arteries of anesthetized C57BL6/J mice were exposed, fluorescently labeled (cell nuclei and cytoplasm), and imaged. Blood pressure and respiratory signals were used to generate a trigger pulse for acquiring single images at a stable moment in the cardiac and respiration cycle. Image acquisition was accelerated by reducing the number of scanned pixels or reduction of pixel dwell times. Filtering in Fourier domain was examined to improve overall image quality.

Timing of the trigger moment at diastole in the cardiac cycle combined with acquisition speeds of 4 Hz or higher resulted in stable images with reduced motional artifacts. Subsequently acquired images contained a similar and stable image of the artery. We have shown the feasibility of stable imaging of large arteries *in vivo* using TPLSM triggered on cardiac and respiratory cycle. This method creates new opportunities for *in vivo* studying of arterial wall properties at subcellular level.

PMI5

NEW MICROSCOPIC TECHNIQUE FOR REAL-TIME VISUALISATION OF THE MICROCIRCULATORY SYSTEM IN THICK INTRAVITAL TISSUES

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Capillaroscope has been used widely for cutaneous capillaries examination in microcirculatory studies. However, current technologies are still incapable of imaging nailfold microvessels non-invasively with adequate magnification and clarity concurrently. This study aimed to identify the main source of this problem and subsequently, to develop an improved image-acquiring technique. We proposed that blurring is mainly caused by the presence of optical background noise (OBN) in the field of view. Hence, it was hypothesized that as the OBN is reduced or eliminated in the field of view, image clarity will be enhanced. The first study investigated the source of blurring by comparing side illumination using 'Alpha Elusion Approach'

and top illumination using conventional microscope. Results showed that side illumination gave clearer images due to reduced OBN in the field of view. The second study examined the influence of increasing magnification on clarity. Increasing numerical aperture for high magnification imaging introduced increased blurring and this could be explained by higher noise-to-signal ratio in the field of view. In conclusion, the key to attaining clear images is to ensure absence of OBN in the field of view. This concept could be applied on other biomedical imaging systems to visualise organs under thick tissues non-invasively.

PMI6

NOVEL METHOD OF BLOOD FLOW REAL-TIME VISUALIZATION

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The real-time blood velocity measurement over large areas is important to provide an easy visualization of blood vessels and an accurate mapping of blood flow. Laser Speckle Contrast Imaging (LSCI) or Laser Speckle Contrast Analysis (LASCA) is a method capable of simultaneous visualization of large area of blood vessels. This method utilizes the so-called light speckle effect, where the coherent light is scattered by RBC's. The LSCI technique is used extensively for investigating blood flow, but two fundamental problems have arisen in using it; a) lack of ability to measure an absolute blood velocity values and b) reduced sensitivity for low values of blood flow. Both disadvantages strongly impair the capability to investigate subtle changes, occurring in capillary flow. The new blood circulation visualization system has employed a LSCI method with additional proprietary algorithm allowing a combination of the full-field real-time blood flow visualization with absolute blood velocity measurement and particularly increased sensitivity to the low values of blood flow. The modified LSI method and newly developed algorithms have been tested in-vitro and in-vivo in animal model. The blood flow has been reduced artificially and visualization capabilities of the conventional LSCI method and the newly modified system have been compared. The new method demonstrated strong capabilities in tracking of very subtle blood flow variations.

PMI7

FFT ANALYSIS OF THE INTRAVITAL FLUORESCENCE MICROSCOPY SIGNAL OF SUBCUTANEOUS BLOOD VESSEL DIAMETER CHANGES INDUCED BY LOW FREQUENCY-ELECTROMAGNETIC FIELDS

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In previous our investigations, changes in arterioles in vivo were examined in mice exposed to low frequency-electromagnetic fields (EMF), estimation of the changes of blood vessel diameter was provided by means direct, intravital microscopy.

Changes in blood vessel diameter have a specific spectrum. Calculating the spectrum of the blood vessel diameter changes by FFT could allow us to be more accurate in estimation of specific changes in blood flow, blood vessels structure, hormonal, vegetative nervous system and metabolic activity in surrounded tissue; all these issues are involved in formation of the complex signal of blood vessel diameter changes.

Conscious BALB/c mice (n=34) assigned to 1 of 4 groups were exposed to EMF for 10min: at 10, 16 and 50Hz (28mT), or no exposure (sham). All investigated animals were with Dorsal Skin-fold Chamber-DSC, implanted mice were injected with FITC-Dextran-250, 2.5% in PBS, via caudal vein. Epi-illumination of arterioles (diameter 45-80 microm) was monitored continuously over 33min, which included: 10min pre-, 10 min EMF-exposure, and 10 min post-exposure. In vivo alterations in vasomotion were measured using a high-speed digital-video recording and image enhancement system, employing an edge-gap detection algorithm (389ms sampling time), to calculate arteriole diameters. Captured signal images were filtered and amplified to eliminate background fluorescence noise (by Savitzky-Golay algorithm). Changes in frequency band magnitudes were determined by FFT (Fourier coefficients) and wavelet analysis. We analyzed and compared amplitudes of five harmonics, which are components of the complex signal; we compared amplitude of oscillation of each harmonic at different conditions low frequency EMF exposure with frequencies 10, 16 and 50Hz (28mT), or no exposure (sham).

Sham-exposed mice exhibited no significant changes in vessel diameters during the 33min evaluation period. However, 16Hz EMF significantly increased vasodilatation post-exposure, compared with pre- and exposure periods (up to 20 microm). No significant change with 10Hz or 50Hz exposure on vessels was seen throughout the monitoring, using Friedman's Chi-Square analysis. This on-line, automated and real-time methodology enables improved, advanced analysis of single frequency components among the compound signals monitored in vasomotion, toward better understanding signal transduction pathway interactions/factors related to the mechanisms of microvascular regulation and change.

PMI8

MICROCIRCULATION DURING CARDIAC ARREST AND CARDIOPULMONARY RESUSCITATION – SUBLINGUAL SIDESTREAM DARK-FIELD (SDF) IMAGING

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Sidestream dark-field (SDF) imaging provides unique opportunity to observe and assess functional capillary density (FCD) and microvascular flow index (MFI) during cardiac arrest and cardiopulmonary resuscitation (CPR) in relation to therapeutic interventions. Six female domestic pigs were anesthetized, mechanically ventilated and instrumented. Microcirculatory status of sublingual mucosa has been recorded at the baseline, immediately after ventricular fibrillation (VF) induction (t=0,5min), after initiation of chest compression first without (t=10min) and finally (t=18min) with mechanical ventilation (MV). Immediate effect of epinephrine (t=30min) and time to microcirculatory arrest after termination of CPR (t=60min) have been studied. Microcirculatory parameters have been evaluated off-line, systemic hemodynamics has been monitored throughout the study. Dramatic decrease of FCD and MFI has been detected during VF, surprisingly complete stop-flow was never observed. Significant increase of FCD and MFI was associated with chest compression, further improvement of FCD and MFI was detected after initiation of MV. Epinephrine administration resulted in an extreme reduction of FCD and minor reduction of MFI. An average time to microcirculatory arrest when CPR was terminated and no flow was detected varied from 250 to 350 seconds. Except for the onset of VF, microcirculatory flow correlated with macrohemodynamics. VF at an early phase does not lead to complete microcirculatory arrest, early start of chest compression is essential to keep the best possible microcirculatory flow. Supported by the Research project MZO 00179906.

Thursday, August 28th
Ischemia and reperfusion
Poster Session – PIR

PIR1

DIETARY NITRITE SUPPLEMENTATION REDUCES INFARCT SIZE IN A MURINE MODEL OF MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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Nitrite has emerged as an endogenous storage molecule of Nitric oxide (NO) with potential therapeutic implications for cardiovascular disease. Steady-state levels of nitrite are derived in part from dietary sources; therefore, we investigated the effects of dietary nitrite supplementation and deficiency on NO homeostasis and on the severity of myocardial ischemia/reperfusion (MI/R) injury. Mice fed a standard diet with supplementation of nitrite (50mg/L) in their drinking water for 7 days exhibited significantly higher plasma levels of nitrite, significantly higher heart levels of nitrite and NO metabolites and displayed a 48% reduction in infarct size following MI/R. Nitrite was found to be consumed during the ischemic phase with a concomitant increase in nitroso/nitrosyl products in the heart and plasma, which were restored to baseline levels by 30 min of reperfusion. Mice fed a diet deficient in nitrite for 7 days exhibited significantly diminished plasma and heart levels of nitrite and NO metabolites and a 59% increase in infarct size following MI/R. These data demonstrate the influence of dietary nitrite intake on plasma and myocardial levels of nitrite and NO and illustrates the cytoprotective effects of dietary nitrite supplementation and consequences of nitrite deficiency on the pathophysiology of MI-R injury. This work was supported by the National Institutes of Health (HL-060849-08 to D.J.L. and F32 DK 077380-01 to J.W.C.) and the American Diabetes Association (7-04-RA-59 to D.J.L.).

PIR2

NEUROTROPHIN-3 (NT-3) PROMOTES REPARATIVE NEOVASCULARIZATION AND BLOOD FLOW RECOVERY IN A MOUSE MODEL OF LIMB ISCHEMIA

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Neurotrophins regulate neuronal survival and regeneration. Non-neural functions of neurotrophins include angiogenesis promotion by NGF and BDNF and NT-3 involvement in heart development. The possibility that NT-3 promotes angiogenesis has not been evaluated yet. Therefore, we investigated the pro-angiogenic potential of NT-3 by adenovirus(*Ad*)-mediated-*NT-3* over-expression in a mouse model of limb ischemia. We preliminary found that capillaries and arterioles endothelial cells(EC) express trkC in skeletal muscles, thus suggesting their responsiveness to NT-3.
Limb ischemia was induced by left femoral artery occlusion on anaesthetized CD1 mice and *Ad-NT-3*(10^8p.f.u.) was injected in ischemic adductors. At day3 post-ischemia the underpinning molecular pathways were analysed on muscles. Blood flow(BF) recovery to the ischemic foot was measured by Colour Laser Doppler on day7 and 14. Histology and IHC analyses of muscles were performed after 3(EC proliferation) and 14days (capillary and arteriole densities) post-ischemia.
Ad.NT-3 induced phosphorylation of trkC receptor, Akt and eNOS($P<0.05$ for comparisons vs. control), while it did not change VEGF-A expression. *Ad.NT-3* increased the number of proliferating EC($P<0.05$) and the density of both capillaries($P<0.01$) and arterioles($P<0.05$) in ischemic adductors and it improved BF recovery($P<0.05$ vs. control on both day7 and 14).
NT-3 could be considered as a candidate factor for treatment of ischemic vascular disease.

PIR3

MICROCIRCULATORY CHANGES AFTER AUTOLOGOUS BONE MARROW-DERIVED STEM CELL TRANSPLANTATION (ABSCT) IN PATIENTS WITH CHRONIC CRITICAL LIMB ISCHAEMIA

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Autologous bone marrow-derived stem cell transplantation (ABSCT) is an arising therapy modality with an option of building new blood vessels through endothelial stem and/or progenitor cells in patients with unreconstructable critical limb ischaemia (UCLI).
Five patients with UCLI were treated by ABSCT. CD34+/CD133+ cell counts were determined in aspirated bone marrow, CD34+ cells were isolated by magnetic separation and collected into a 10 ml sample. The cell suspension was administered by local intramuscular injections (0.5-1.0 ml injections in the musculus gastrocnemius). The follow-up based on clinical (rest

pain, walking distance without pain, changes of non-healing ulcers, ABI) and laboratory (angiography, Color- and laser-Doppler scan, TcPO2 measurement and endothel function test) parameters was documented and analyzed.
Improvement of pain and walking distance was observed in all five cases. Before and 6, 20, 52 weeks after transplantation the TcPO2 changed on the foot from 18.80 to 16.78; 23.83; 37.5 mmHg, and on the calf from 36.66 to 31.25; 45.00; 37.26 mmHg. The laboratory parameters improved after 3 months. No adverse events were observed.
ABSCT is safe and effective in patients with UCLI. Multicentric, controlled clinical trials are required to confirm these preliminary results.

PIR4

REGULATION OF MICROCIRCULATION IN THE DEVELOPING CALLUS OF THE RABBIT.

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Previous studies have demonstrated a rapid neural and vascular ingrowth into the developing callus in the rabbit tibia. We wanted to study the maturation/development of the regulation of microcirculation in the same animal model. A 3-mm gap osteotomy was carried out and followed by plate fixation in the shaft of the tibia. Linear flow velocity in the callus and in the ipsilateral femur was assessed by laser-Doppler flowmetry, and systemic arterial blood pressure was monitored (Experimetria Ltd. Budapest, Hungary). Blood flow reactions were elicited by i.a. applied bolus injections of vasoactive substances [Epinephrine (Epi), CGRP, SP, SNP]. Duration of responses and peripheral resistance were calculated. Measurements were carried out on the 10th and 15th postoperative days, respectively. Linear flow velocity in the femur was always higher than in the normal tibia or in the callus and it was also higher on the 15th than on the 10th p.o. days in the callus. With the exception of Epi flow changes on the 10th p.o. day followed the changes of the perfusion pressure. Stimulation of α_1 receptors (Epi) elicited vasoconstriction on the 10th day and this reaction was identical to that in the femur on both days, however, receptor binding lasted longer in the callus than in the femur. Flow changes on day 15 still followed pressure changes in the callus after CGRP, SP, and SNP. These vasodilators decreased peripheral resistance in the femur. Thus, there is rapid vascularisation of the developing callus in the rabbit tibia; α_1 receptors appear on the vessels already on the 10th p.o. day, however, the development of counter-regulatory effects of vasodilators is still not complete on day 15.
Grants: Hungarian Sci. Res. Funds/OTKA -K67984

PIR5

AMELIORATING EFFECTS OF NOTOGINSENOSIDE R1 ON ISCHEMIA-REPERFUSION-INDUCED MICROVASCULAR DAMAGE IN RAT MESENTERY-IN VIVO MICROSCOPIC ANALYSIS

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Notoginsenoside R1 (R1) is one of the major active ingredients of *Panax Notoginseng*, which is a Chinese medicine for clinical treatment of vascular diseases. The aim of this study is to examine the effects of R1 on ischemia/reperfusion (I/R)-induced rat mesenteric microcirculatory injuries. Under sodium pentobarbital anesthesia, the mesenteric microcirculation in the ileocecal portion of a male Wistar rat was observed through an inverted-type intravital microscope with a charge-coupled device or a silicon-intensified target camera. I/R was conducted by a ligation of the mesenteric artery and vein for 10 min and a subsequent release of the blood flow occlusion. We measured the venular diameters, numbers of adherent leukocytes, FITC-albumin leakage, dihydrorhodamine 123 (DHR) fluorescence and mast cell degranulation. R1 (5 mg/kg/hr) was continuously infused from 10 min before the ischemia. Pretreatment with R1 reduced peroxide production, leukocyte adhesion in the venular wall, FITC-albumin leakage from venules, as well mast cell degranulation in perivascular region, all of which were induced by I/R.
In conclusion, R1 ameliorates the I/R-induced microcirculatory disturbances by inhibiting peroxide production, leukocyte adhesion in the venular wall and mast cell degranulation.

PIR6

HAEMATOPOIETIC STEM CELL RECRUITMENT TO HEPATIC MICROCIRCULATION FOLLOWING ISCHAEMIA-REPERFUSION INJURY IS DEPENDENT ON THE $_{4\ 1}$ INTEGRIN

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Hematopoietic stem cells (HSCs) migrate to injured liver and may aid tissue repair. However, no studies have identified molecular mechanisms underlying this recruitment. We utilised fluorescent intravital microscopy (IVM) to determine the roles of $_{4\ 1}$, CD31, CD44 and CD18 in promoting HSC recruitment after hepatic ischaemia-reperfusion (IR) injury *in vivo*. Hepatic ischemia was induced for 90 min in anaesthetised C57BL/6 mice (ketamine/xylazime). CFSE-labelled HSCs ($1x10^6$ HPC-7s; ia) were administered post-reperfusion and IVM observations made for 60 minutes. Some HPC-7s were pre-treated with function blocking antibodies to specific adhesion molecules. IR injury significantly increased HPC-7 adhesion (13.3+/-1.7 vs 3+/-1.0 at 30 mins; $p<0.05$). HPC-7 pre-treatment with an $_{4\ 1}$ blocking antibody significantly reduced adhesion compared to IgG control pre-treated cells (5.5+/-1.5 vs 15.5+/-1.9 at 55 mins; $p<0.001$). Pre-treatment with CD31, CD44 and CD18

antibodies failed to reduce adhesion at any time point. Using an *in vitro* flow based adhesion assay, the role of $_{4\ 1}$ integrin was confirmed since firm adhesion and migration was significantly ($p<0.001$) reduced, but rolling increased, on murine cardiac endothelial cells. Our novel data indicates a central role for $_{4\ 1}$ in mediating HSC recruitment, indicating similar adhesion molecules to those used by mature leukocytes are also used by HSCs.

PIR7

TLR2 AND TLR4 DIFFERENTIALLY MEDIATE POSTISCHEMIC LEUKOCYTE TRANSMIGRATION AND VASCULAR PERMEABILITY

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Ischemia-reperfusion (I/R) activates innate immunity involving toll-like receptors (TLR), in particular TLR2 and 4. Leukocyte migration and vascular permeability are critical events leading to postischemic tissue damage. Here, we investigated the role of TLR2 and TLR4 for leukocyte rolling, adhesion, transmigration, interstitial migration, and vascular permeability during I/R in murine cremaster muscle using intravital microscopy. Sham-operated mice without ischemia and wild-type, TLR2-/-, and TLR4-mutant mice undergoing I/R (30/120 min) were analyzed (n=7). In TLR2-/- and TLR4-mutant mice, I/R-induced leukocyte rolling and adhesion were comparable with those in wild-type mice. The number of transmigrated leukocytes was increased in wild-type mice upon I/R as compared to sham. In contrast, leukocyte migration was significantly attenuated in TLR2-/-, but not in TLR4-mutant mice. Motility and polarization of interstitially migrating leukocytes did not significantly differ in TLR2-/- and TLR4-mutant mice as compared to wild-type. I/R-induced vascular leakage was significantly lower in both TLR2- and 4-deficient than in wild-type mice. Thus, we suggest that TLR2 and TLR4 have shared and distinct functions during I/R. TLR2 mediates both postischemic leukocyte transmigration and vascular leakage, whereas TLR4-triggered permeability is dissociated from leukocyte transmigration. Postischemic leukocyte rolling, adhesion, and interstitial migration, however, are unaffected by deficiency of both receptors. This study was supported by EU NoE “MAIN” (LSHG-CT-2003-502935)

PIR8

EFFECT OF MESENTERIC LYMPH REPERFUSION ON SUPERIOR MESENTERIC ARTERY OCCLUSION SHOCK RATS

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Duplicate the SMAO shock model by superior mesenteric artery occlusion for 1h then reperfusion for 2h, named “mesenteric lymphatic occlusion and then reperfusion” for MLR. The mean arterial pressure (MAP), organs histopathological and functional changes were observed. The results showed that the 24h survival rates of Sham, MLR, SMAO group (100%, 83.3%, 66.7%, respectively) were higher than MLR+SMAO group (0%). The MAP of SMAO group was higher than MLR+SMAO and Sham group at multi-time points after clamping; the MAP of SMAO group at multi-time points was decreased than MLR, Sham group, the MLR+SMAO group was decreased than SMAO, MLR, Sham group at all time points after reperfusion. The AST, ALT, BUN, Cre, LDH-1, CK-MB in MLR+SMAO group were higher than Sham, MLR, SMAO group, and that of SMAO group were higher than Sham, MLR group. Pathomorphology of lung, kidney, myocardium, liver showed that inflammation, congestion, degeneration and necrosis were found in MLR+SMAO group, only mild damage was found in MLR, SMAO group. Our study showed that MLR could facilitate important organ dysfunction and damage of SMAO shock, therefore, intestinal lymphatic pathway played an important role in the pathogenesis of SMAO shock. The work was supported by NSFC(30770845) and NSFHebei(C2008000503).

PIR9

TONIC INHIBITORY CONTROL OF THE MESENTERIC MICROCIRCULATION BY THE MELANOCORTIN TYPE 3 RECEPTOR

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Melanocortin peptides are endowed with anti-inflammatory properties; their actions are mediated by a subgroup of G-protein coupled receptors (MCRs). Here we addressed the patho-physiological impact of melanocortin peptide receptor 3(MC3R) after an ischaemia-reperfusion (I/R) procedure.
C57BL/6 (WT), MC3R null or MC1R mutant mice were anaesthetised and surgically prepared; ischaemia was produced by occlusion of the superior mesenteric artery (SMA) for 35min; reperfusion was allowed for 90min. Leukocyte-endothelial interactions were monitored by intravital microscopy, quantifying cell rolling (µm/s), cell adhesion and emigration. Sham animals underwent the same surgical procedure without clamping. In some animals, the MC3R agonist $dTrp^8$- -MSH (10µg/mouse) was given i.v. before SMA clamping. MC3R null mice displayed marked augmentation of cell adhesion and emigration (two-fold increase *vs.* WT mice, $P<0.05$, n=6), whereas no changes in cell rolling were evident. MC1R mutant mice showed no difference from WT. Pharmacological treatment of animals with $dTrp^8$- -MSH produced marked attenuation (>60%) of cell adhesion and emigration ($P<0.05$; n=6).
Collectively these data indicate the existence of a tonic inhibitory signal provided by MC3R in the mesenteric

microcirculation. Therefore, therapeutic targeting of MC3R may be beneficial for the treatment of acute I/R-related injury, e.g. aorta aneurysm, and coronary disease. Supported by the British Heart Foundation (FS/05/078/19406)

PIR10

HETEROGENEITY OF PERFUSION AND FUNCTION CAUSED BY VASCULAR NETWORK STRUCTURE AND ADAPTATION

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In the heart and other tissues, perfusion and metabolic activity exhibit significant, stable and positively correlated spatial heterogeneity, raising the question of causal relationships involved. Here, the hypothesis is evaluated that observed heterogeneities are mainly caused by inevitable structural heterogeneity of terminal vascular beds and are modified by adaptation of vascular diameter in response to metabolic signals and adaptation of local oxygen demand to oxygen availability. A mathematical simulation based on experimental data for microvascular networks is used. Heterogeneity of blood flow and of oxygen levels cannot be eliminated by increasing the metabolic sensitivity for diameter adaptation. Adaptation of demand increases overall oxygen extraction and thus functional capacity, and establishes a correlation between local oxygen demand and flow rate, which is not observed if only intrinsic heterogeneity of oxygen demand is assumed. These findings suggest that observed heterogeneities in flow and metabolic activity are caused by the topological and angioarchitectural heterogeneity of vascular networks and not by intrinsic heterogeneity of local metabolic demand.

PIR11

RAW VALUES RATHER THAN BASELINE-DEPENDENT EXPRESSION OF POST-OCCLUSIVE REACTIVE HYPEREMIA

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Different techniques are commonly used to assess skin microvascular function, among which post-occlusive reactive hyperemia (PORH). However, data expression remains an issue as there is no consensus. Data can be expressed as peak raw values or as a function of baseline flux, i.e. peak/baseline percentage increase (P/B), peak minus baseline (P-B) or area under the curve (AUC). Our objective was to compare PORH expressed as peak raw value, P/B, P-B or AUC in different baseline conditions. We performed a 5-minute occlusion of the brachial artery and measured skin blood flow of the forearm with laser-Doppler flowmetry in 8 healthy volunteers. Then, we repeated the measurement after iontophoreting sodium nitroprusside (20 seconds 100 microA, twice) to elevate baseline. Median peak cutaneous vascular conductance (interquartile) without and with iontophoresis were 4.7 (3.3) and 3.3 (2.8) mV/mmHg, respectively (NS, Wilcoxon test). P/B were 585 (206) and 143 (81)% (p=0.01), P-B were 3.9 (3.1) and 1.1 (1.2) mV/mmHg (p=0.01) and AUC were 866 (932) and 231 (604) PU.s (p=0.01). Baseline-dependent expressions of PORH were highly variable within the same subject, whereas peak cutaneous vascular conductance was reproducible. Peak raw values should be used rather than P/B, P-B or AUC to express PORH results.

PIR12

SEVERE HYPOXIA SUPPRESSES CARDIAC AUTONOMIC FUNCTION AND VASCULAR HEMODYNAMIC CONTROL DURING POSTURE CHANGE AND VALSALVA EXERTION

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This study was to investigate how various hypoxic interventions affect cardiovascular functions during posture change and Valsalva exertion. Ten healthy sedentary men were randomly exposed to 12% (severe hypoxia, SH), 15% (moderate hypoxia, MH), and 21% (normoxia, N) O_2 for one hour in a normobaric hypoxia chamber. An impedance plethysmography was used to measure arterial and venous hemodynamics, whereas a spectral analysis of heart rate variability (HRV) was employed to determine cardiac autonomic function. The SH exposure 1) decreased the ankle-brachial index, which was accompanied by a decrease in arterial reactive hyperemia and an increase in venous flow resistance; 2) decreased total power and high frequency (HF) as well as increased low frequency (LF) and LF/HF in HRV; 3) lowered changes of time and frequency domains in HRV induced by posture change; and 4) reduced blood pressure and hart rate responses to Valsalva exertion. However, no significant change occurred in resting and physical stress-mediated the HRV and vascular hemodynamic values following either MH or N exposure. We conclude that acute hypoxia impacts cardiovascular functions, with reactions determined by intervening O_2 concentration. The SH, but not the MH down-regulates cardiac autonomic and vascular hemodynamic responses to posture change and Valsalva exertion.

PIR13

FACTORS CONTRIBUTING TO HEMATOPOIETIC STEM CELL RECRUITMENT TO MURINE CREMASTER MICROCIRCULATION

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Hematopoietic stem cells (HSCs) may be utilised therapeutically for a number of degenerative diseases. However, it is unknown which adhesion molecules and cytokines are required for HSC homing to extramedullary tissues, and whether the kinetics of recruitment, are similar to mature leukocytes. This was investigated in injured and cytokine stimulated cremaster muscle intravitally. The cremaster underwent ischemia-reperfusion (IR) injury or was stimulated with IL-1 (4 hrs; 12.5ng/200 l) in anaesthetised C57BL/6 mice. CFSE-labelled HSCs ($1x10^6$ HPC-7 cells) were administered post-reperfusion or post-IL-1 . Some HPC-7s were pre-treated with a function blocking CD18 mAb (GAME-46). HPC-7 rolling and static adhesion was significantly increased in post-capillary venules (PCVs) in both IR injured ($p<0.05$) and IL-1 stimulated ($p<0.05$) animals compared to controls. Significant ($p<0.05$) capillary recruitment was also observed with IL-1 . The CD18 mAb significantly inhibited IR injury and IL-1 induced PCV and capillary adhesion but not rolling. FACS demonstrated that HPC-7 surface expression of CD18 was not increased in response to IL-1 incubation. Our novel results illustrate that HSCs can indeed roll and firmly adhere to injured and cytokine stimulated tissue in a manner similar to mature leukocytes. Furthermore, this phenomenon also occurs predominantly in PCVs and shares similar adhesion molecules to mature leukocytes. Funded by the Royal Society .

Thursday, August 28th
Vascular tone control
Poster Session – PTC

PTC1

DEVELOPMENTAL ADAPTATIONS OF VASCULAR RESPONSES TO CONTRACTILE AND DILATORY AGENTS IN THE OVINE SPLANCHNIC ARTERIAL TREE

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Ability to redistribute blood depends on precise control of splanchnic circulatory bed. Developmental changes in blood supply to omentum, particularly arterial reactivity remains unknown. Hence, we examined arterial responses to contractile and relaxant agonists during development from foetal to adult life. Omental arteries from term foetuses, lambs (day 1, 1-6 weeks old, 3-6 months) and adults were isolated and mounted on a small vessel wire myograph. Vascular responses to noradrenaline (NA), thromboxane mimetic (U46619), potassium (K^+), acetylcholine (ACh) and bradykinin (BK) were determined. Sensitivity (EC_{50}, $P < 0.05$) of foetal and lamb arteries to NA was lower compared to adult vessels. Maximal arterial contractile responses of lambs at 1 to 6 weeks of age were different from other groups. Responses of foetal and lamb arteries to U46619 were non-existent, but fully developed in the adult vessels. Moreover, foetal and lamb vessels demonstrated a decreased sensitivity ($P < 0.05$) and reduced maximum response ($P < 0.01$) to increasing concentrations of depolarising potassium as compared to adult vessels. Vasorelaxant responses to ACh and BK were fully developed at all ages, and no significant difference was detected between groups. To conclude, receptor-mediated and non-receptor contractile systems undergo substantial changes during early development.

PTC2

ROLE OF CYCLOOXYGENASE (COX) METABOLITES IN TRYPTAMINE-ELICITED VASOPRESSOR RESPONSES IN RAT ISOLATED MESENTERIC ARTERIAL BED

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Tryptamine (Trp), an indolealkaloid, is a trace amine synthesised in central and peripheral tissues, found in various food items and released by microflora in the gastrointestinal tract. Trp produces vasopressor effects, and here we test for the possible involvement of the arachidonic acid cascade in Trp-mediated vasoconstrictor responses. Rat isolated mesenteric arterial bed was perfused with Krebs'- bicarbonate solution at 37ºC, gassed with carbogen, pH 7.4. Trp evoked dose-dependent vasoconstriction (pD_2: 105.5 ± 17.5 nmol / 100µL, maximal response: 40.3 ± 3.6 mmHg). Pre-treatment with indomethacin (10 µM, COX-1 inhibitor) essentially abolished Trp-derived contractions, but nimesulide (10 µM, COX-2 blocker) reduced Trp-vasoconstrictions from 50.3 ± 0.9 to 23.2 ± 2.5 mmHg. $PACOCF_3$ (10 µM, phospholipase A_2 inhibitor) produced no changes in Trp-contractions. These Trp-evoked contractions were markedly suppressed by AH 6809 (10 µM, EP-receptor blocker) and were not affected by ICI 192,605, a thromboxane A_2 receptor antagonist (1 µM, TP receptor). Tranylcypromine (10 µM, prostacyclin synthase inhibitor) elevated contractions at low but not high Trp-concentrations. In conclusion, PGE_2, possibly via EP_1 receptors, is potential mediator of vasoconstrictor responses to Trp, which may play a role in regulating mesenteric blood flow. [MAA supported by a Wellcome Trust fellowship]

PTC3

TEMPERATURE SUBSTANTIALLY MODULATES THE BASAL TONE OF ARTERIOLES

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The role of hemodynamic forces, metabolic factors, neuronal and hormonal mechanisms in regulation of vascular tone has been well established. Clinical observations and temperature-treatments emphasize the importance of temperature on tissue blood flow, yet the potential role of temperature in regulation of microvascular resistance is less known. We hypothesized that changes in temperature have a direct effect on the diameter of microvessels. Thus in isolated coronary arterioles changes in diameter were measured during increases and decreases of bath temperature (from 37°C to 15°C and from 37°C to 42°C). We have found that progressive reduction of temperature increased the diameter (123±6 µm, at 37 °C vs. 187±8 µm, at 15°C, p<0,05), whereas increases of temperature reduced the diameter (88±10 µm, at 42°C, p<0,05) of vessels. Simultaneous administration of L-NAME and indomethacin significantly inhibited the increase in diameter induced by reducing the temperature (61±4 % vs. 45±5 %, at 15°C), but did not effected the decrease in diameter induced by increasing the temperature (-33±5 % vs. -37±4 %, at 15 °C). In addition, the K-ATP channel blocker glibenclamide reduced the dilation to low temperature (61±4 % vs. 51±4 %, at 15°C). These findings suggest that rising temperature substantially increases, whereas reduction of temperature decreases the resistance of arterioles. The mechanisms involved likely include endothelial factors and K-ATP channels. Thus local changes in temperature could have substantial effects on tissue blood flow. Further

elucidation of cellular mechanisms would greatly increase our understanding regarding the temperature dependent regulation of microcirculation in physiological and pathological conditions.
Grants: AHA NE Aff. 0555897T; Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006.

PTC4

ENDOTHELIN-1-MEDIATED CORONARY VASOCONSTRICTION IS ATTENUATED DURING EXERCISE BY A REDUCED ET-RECEPTOR SENSITIVITY

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Previous data have demonstrated that coronary exercise hyperemia involves the withdrawal of endothelin-1 (ET-1)-mediated vasoconstriction in swine. This study examined whether this withdrawal is due to an exercise-induced reduction in coronary ET-1 receptor sensitivity and/or production from its inactive precursor big-ET-1 by endothelin converting enzyme.
The hemodynamic responses to graded infusion of ET-1 (10 and 20 pmol/kg/min) and its precursor big-ET-1 (10, 20 and 40 pmol/kg/min) were compared both at rest and during exercise at 3 km/h in chronically instrumented Yorkshire swine.
Infusion of exogenous ET-1 increased coronary vascular resistance (CVR) by 29±14% (low dose) and 89±13% (high dose) from baseline at rest and by 15±4% (low dose) and 55±7% (high dose) during exercise. Infusion of Big-ET-1 increased CVR by 0±10%, 18±3%, 67±10% (for 10, 20 and 40 pmol/kg/min) from baseline at rest and by 8±1%, 54±5% (10 and 20 pmol/kg/min) during exercise.
Since exercise resulted in a similar attenuation of the vasoconstrictor effect of ET-1 and big-ET-1, it is unlikely that the production of ET-1 from its precursor big-ET-1 is affected by exercise. Therefore, we conclude that the attenuation of the vasoconstrictor effect of ET-1 during exercise is mediated by a reduction in receptor sensitivity.

PTC5

IMPORTANT ROLE OF IK AND SK IN EDHF-TYPE DILATIONS IN ISOLATED SMALL ARTERIES

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Endothelial release of NO, PGI2 and the endothelium derived hyperpolarizing factor (EDHF) mediates vessel dilation upon agonist stimulation. EDHF-type dilations may require calcium activated potassium channels (IK, SK). We investigated acetylcholin-induced (ACh) dilations in preconstricted (norepinephrine) conducting (CA, A. femoralis) and small arteries (SA, gracilis artery) of wildtype (wt) and IK-deficient (IK-/-) mice to analyse the relevance of IK and SK. Isolated vessels were mounted under isometric conditions.
Dilations were equal in CA of wt and IK-/- under control conditions and abrogated after inhibition of NOS and COX (L-NA and indomethacin, L+I). In wt SA dilations were not reduced after L+I indicating the importance of EDHF. Additional incubation with the SK-blocker Apamin (Apa) significantly reduced dilations (from 80±7 to 48±7%; ACh: 1microM). In contrast to wt, dilations were reduced significantly by L+I in IK-/- (IK-/-: 61±4 vs. wt: 80±7%). The remaining response was abrogated after incubation with Apa.
Dilations are mediated by NO in CA. However, EDHF-type dilations predominate in small arteries. Our data suggest a critical role of calcium-activated potassium channels IK and SK therein. Moreover, IK and SK are involved equally in EDHF-type dilations and cannot compensate for each other.

PTC6

CX40 IS REQUIRED IN EDHF-TYPE DILATIONS IN VITRO BUT NOT IN VIVO IN SMALL ARTERIES

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Vessel dilations upon acetylcholine (ACh) are induced by endothelial release of NO, PGI2 and an endothelium derived hyperpolarizing factor (EDHF). Myoendothelial gap junctions (MGJ) composed of connexins (Cx) may be involved in EDHF-type dilations by providing a direct pathway for charge transfer from endothelium to smooth muscle. To elucidate the role of MGJ in EDHF-type dilations we performed in vitro and in vivo studies in preconstricted (NE, 1microM) small arteries (SA, gracilis artery) of wildtype (wt) and Cx40-deficient (Cx40-/-) mice. ACh-induced dilations in small arteries were reduced after inhibition of NOS and COX (L-NA and indomethacin, L+I, from 70±6 to 40±6%) and abrogated under high K+ (50mM) conditions. In Cx40-/- vessels, ACh-dilations were significantly smaller under control conditions (Cx40-/-: 42±11 vs. wt: 70±6%) and abrogated after L+I (-8±15%). However, if the same artery was studied in vivo or under isobaric conditions ACh-dilations were similar in wt and Cx40-/- (~80%) and unaffected by L+I. EDHF plays a central role in ACh-induced dilations in small arteries and is more powerful in vivo and under isobaric conditions. This may represent a distinct EDHF, which is lost in isometric systems and substituted under these conditions by an EDHF that relies on MGJ and Cx40.

PTC7

POTENTIAL ROLE OF HYDROGEN PEROXIDE IN MEDIATION OF FLOW-INDUCED RESPONSES OF ARTERIOLES

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Flow-induced dilation of arterioles plays an important role in the regulation of tissue perfusion, yet the cellular signaling mechanisms are still not completely elucidated. Recently, it has been suggested that H_2O_2 mediates flow-induced dilations of human atrial coronary arterioles and that micromolar concentrations of H_2O_2 elicit biphasic response of skeletal muscle arterioles. Thus, we hypothesized that H_2O_2 plays a role in the development of flow-induced dilations of skeletal muscle arterioles. Isolated rat gracilis muscle arterioles (~ 140 m) of male Wistar rats were used. First, responses to intraluminal administration of H_2O_2 ($3x10^{-5}$-10^{-4} M) were obtained in the absence and presence of extraluminally administered PEG-catalase. Next, the effects of stepwise (1) or one great, sudden step (2) increase in intraluminal flow were investigated in the absence, or presence of extraluminal PEG-catalase. H_2O_2 applied intraluminally elicited biphasic changes in the diameter: after initial constriction (max.: 22 3%), substantial dilation (max.: 43 5%) developed, both of which were inhibited by extraluminal administration of PEG-catalase (250 U/ml). Stepwise increases in flow elicited dilations (max: 39 3 m at 40 L/min), which were unaffected by PEG-catalase. In contrast, dilation elicited by one large step increase in flow was inhibited by PEG-catalase. We suggest that in rat skeletal muscle arterioles, H_2O_2 is unlikely to be involved in the development of flow-induced dilation. However, in dilations elicited by great and sudden increases in flow/wall shear stress H_2O_2 may have mediative role. Supported by Hungarian Nat. Sci. Fund, OTKA T48376, OTKA K71591; OTKA K67984 and AHA NE Aff. 0555897T.

PTC8

POTENTIAL ROLE OF HYDROGEN PEROXIDE (H2O2) MEDIATING MYOGENIC RESPONSE OF ISOLATED SKELETAL MUSCLE VENULES

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Our previous studies showed that H2O2 has biphasic changes in diameter of arterioles. Lower concentrations of H2O2 elicited constrictions, whereas higher concentrations after initial constrictions, caused dilations. However, the effect and role of H2O2 in mediation of vasomotor responses in venules are not yet characterized. We hypothesized that H2O2 contributes to the vasomotor responses of skeletal muscle venules. We have found that H2O2 (10-7 - 10-5 M) elicited concentration dependent constrictions (max.: 137+/-8 to 61+/-18 microm., at 10-5 M) and that presence of indomethacin (2.5×10-5 M, 30 min.) inhibited H2O2-induced constrictions of skeletal muscle venules (max.: 135+/-13 microm. vs. 133+/-13 microm, at 10-5 M). Also, compared to the passive diameters (PD, in Ca2+-free Krebs solution) stepwise increases of intraluminal pressure (1-20 mmHg) elicited significant constrictions in isolated venules. Calculation of maximum myogenic tone was 40+/-5% at 7.5 mmHg intraluminal pressure. The presence of catalase (CAT) reduced significantly the pressure-induced development myogenic tone (control: 40+/-5% vs. control+CAT: 60+/-11% of PD, at 5 mmHg pressure). We conclude that in isolated venules H2O2 increases the synthesis of constrictor prostaglandins (probably prostaglandin H2/ thromboxane A2) resulting in constriction and that H2O2 contribute to the development of pressure-induced myogenic response. Thus we propose that H2O2 plays an important role in regulating vasomotor function of venules both in physiological and pathological conditions.Grants: AHA NE Aff. 0555897T; Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006.

PTC9

ACTIVATION OF LARGE CONDUCTANCE CA2+ ACTIVATED K+ CHANNELS IN CORONARY ARTERIOLES OF RATS ON HIGH FAT DIET – POTENTIAL ROLE FOR CAVEOLAE

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We hypothesized that diet-induced obesity modulates BK_{Ca} channel function in coronary arterioles. Male Wistar rats were fed with normal (control) or high fat diet (HFD 60% of saturated fat, for 10 wk). In isolated coronary arterioles (100 µm) acetylcholine (ACh)-induced, EDHF-mediated (presence of L-NAME $2x10^{-4}$ M and indomethacin 10^{-5} M) diameter changes were measured with videomicroscopy. Compared to those of control responses, ACh-induced dilations were essentially preserved in arterioles of HFD rats. The BK_{Ca} inhibitor, iberiotoxin (IBTX), however elicited a greater reduction in ACh-evoked dilation in coronary arterioles of HFD rats, than in control vessels. Correspondingly, dilation to BK_{Ca} opener NS-1619 was significantly enhanced in coronary arterioles of HFD rats, when compared to lean controls. Moreover, we found a reduced immunostainng of the caveolin-1 protein in smooth muscle layer of coronary arterioles of HFD rats. In control arterioles disrupting the caveolae by methyl ß-cyclodextrin caused similar changes in agonist (ACh and NS-1619)-induced dilations, as observed in those of arterioles of HFD rats. We concluded that in coronary arterioles of HFD rats contribution of BK_{Ca} channel activation in ACh-induced, EDHF-mediated dilations is augmented, a mechanism which may be due to the alteration in the function of microvascular caveole.

PTC10

THE ROLE OF ENDOGENOUS CARBON MONOXIDE ON THE BASAL BLOOD FLOW MAINTENANCE IN GINGIVA

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Carbon monoxide (CO) is produced by heme-oxygenase (HO) isoenzymes in the gingiva. The aim of our study was to investigate the effect of CO produced by HO on the gingival resting microcirculation. The gingival blood flow was measured by laser Doppler flowmetry in the upper central papilla before (bsl) and after the application of HO inhibitor (ip. 45 micromol/kg zinc deuteroporphyrin 2,4-bis glycol) for 45 min in anaesthetized rats. The same study was repeated after the blockade of nitric oxide (NO) synthase (1mg/ml L-NAME in tap water ad libitum for 1 week). HO inhibition significantly decreased the gingival blood flow (GBF, bsl vs. 45.min: 570+/-94 vs. 326+/-85 BPU, $p<0.05$) and increased the gingival vascular resistance (GVR, bsl vs. 45.min: 0.23+/-0.04 vs. 0.39+/-0.06 mmHg/BPU, $p<0.05$) without altering the mean arterial blood pressure (MBP). NO deficiency significantly increased the start-up MBP (control vs. L-NAME pretreatment: 108+/-3 vs. 145+/-4 mmHg, $p<0.05$) and GVR (control vs. L-NAME pretreatment: 0.23+/-0.04 vs. 0.35+/-0.04 mmHg/BPU, $p<0.05$) and prevented the vasoconstrictor effect of HO blockade in gingiva. Our data suggest that endogen CO has an important role in basal maintenance of gingival blood flow. The vasodilatory effects of endogen CO is NO dependent. Supports: OTKA T042584 and T049708.

PTC11

CALCIUM DYNAMICS AND VASOMOTION IN ARTERIAL SEGMENTS UNDER ISOMETRIC, ISOBARIC AND ISOTONIC CONDITIONS: A THEORETICAL STUDY

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In vitro, most experimental studies use either an isometric or isobaric setup to analyze the smooth muscle cells calcium dynamics and contraction/relaxation mechanisms on arteries. However in vivo, a blood vessel is continuously submitted to intraluminal pressure variations arising from heartbeat. We use a theoretical model of the smooth muscle calcium and arterial radius dynamics to determine whether results may be considerably different depending on the experimental conditions (isometric, isobaric, isotonic, or cyclic pressure variations). We show that isobaric conditions are the most realistic, as the calcium dynamics is similar under cyclic pressure variations (in vivo like situation) and under a constant pressure. The arterial contraction is less pronounced in isotonic than in isobaric conditions and the vasoconstrictor sensitivity higher in isometric than isobaric or isotonic conditions, in agreement with experimental observations. The model predicts that isometric conditions may generate artifacts like the coexistence of multiple stable states. This multistability may bring about that an existing vasomotion domain is missed in a concentration-response curve to vasoconstrictor, and that this curve is different whether the vasoconstrictor concentration is increased or decreased. We have verified this model prediction experimentally using rat mesenteric arteries mounted on a wire myograph and stimulated with phenylephrine.

PTC12

INTERACTION BETWEEN NITRIC OXIDE SYNTHASE AND CYCLOOXYGENASES 1 AND 2 IN ARTERIOLES OF RAT CREMASTER MUSCLE *IN VIVO*

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The two isoforms of COX have let to the use of specific and/or non-specific inhibitors as anti-inflammatory drugs with controversial cardiovascular effects.
Because any disorder of the microcirculatory regulatory mechanisms can largely affect adequate tissue oxygen supply/demand balance, we studied by intravital microscopy the response of rat cremaster muscle arterioles to selective COX inhibitors. We found in basal conditions that inhibition of COX-1 or COX-2 induced a significant constriction. In contrast, when NOS was inhibited, COX-1 or COX-2 inhibition induced a relative dilation. When NOS was inhibited and ROS degradation activated, COX-1 inhibition did not change the diameter, but COX-2 inhibition induced a significant additional constriction. In the presence of a TP receptor antagonist, and a NOS inhibitor, we found an additional constriction with either COX-1 or COX-2 inhibition.
These results showed that 1/ both COX isoforms have an important role in basal dilatory tone, 2/ presence of NO leads to both COX isoforms favouring dilatory prostanoid synthesis or effect and 3/ this interaction is a ROS-dependent mechanisms.

PTC13

MODELING VASCULAR CONTROL OF HEMODYNAMICS THROUGH CONDUCTED ASCENDING VASODILATION ALONG ARTERIOLAR TREE

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Regulation of convective O_2 supply to capillary beds within organs is accomplished by coordinated changes in upstream arteriolar diameter. To study this regulation system, *in vivo* experiments have been performed using a gas exchange chamber with computer-controlled gas flowmeters to alter the O_2 environment at the surface of rat skeletal muscle. Microvascular responses to sine oscillations in chamber O_2 were determined by measuring RBC velocity (V) and hematocrit (H) in capillaries near the muscle surface. Experiments showed that H changes were delayed by 3-5 seconds relative to velocity and that this delay varied with the animal suggesting a flow response dependent on geometry. To assess how alterations in diameters led to the observed flow response, a model of the arteriolar tree was constructed based on published data, sinusoidal changes in diameter were imposed in one terminal arteriole, and the upstream conduction of

these diameter changes was simulated. A time-dependent model of two-phase (RBCs and plasma) blood flow was then used to calculate hemodynamics in the network and, in particular, examine how the transit time for RBCs to traverse the network could lead to the observed delay in changes in H relative to those in V.
This work is supported by NIH HL-089125, CIHR to CGE and DG, and NSERC to SJMM.

PTC14

ENHANCEMENT OF THE RELAXATION TO POTASSIUM CHLORIDE BY FORSKOLIN IN THE SHEEP CORONARY ARTERY IMPLIES A ROLE FOR CYCLIC AMP.

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We have previously shown that potassium chloride induces concentration-dependent relaxation of the sheep coronary artery on baseline tone. Activation of membrane potassium channels and stimulation of cyclic AMP have been implicated in the mechanism. This study investigates these possibilities. Coronary arteries from sheep hearts were placed in organ chambers (PSS, 95% O2/5% CO2). Baseline tone 1 gram. Concentration-response curves to potassium chloride (KCl) were obtained (12.5mM, 25mM, 72.5 mM and 125mM KCl). Data are percent relaxation from baseline tone (mean+/-SEM). $P<0.05$ for significance. KCl induced concentration-dependent relaxation of the coronary artery (12.5mM: 6.0+/-0.95%; 13.2+/-1.8%; 17.9+/-2.5%; 24.1+/-3.4%). The relaxation was unaffected by ouabain (10^{-5}M). Barium chloride (10^{-4}M) and glibenclamide (10^{-5}M) significantly reduced the KCl relaxation at the highest KCl concentration only (125mM). In the presence of forskolin (10^{-5}M), the stimulator of adenylyl cyclase, the relaxation to KCl was significantly enhanced at the 12.5mM and 25 mM concentration levels (in the presence of forskolin: 12.5mM: 11.4+/-2.2%; 25mM: 20.0+/-1.0%; 72.5mM: 23.7+/-3.9%; 125mM: 27.7+/-3.7%). Forskolin alone did not relax coronary artery. Potassium-induced relaxation of the coronary artery is not directly regulated by activation of potassium channels or Na^+-K^+ ATPase. Marked enhancement of the relaxation by forskolin implicates activation of cAMP in the relaxation process.

PTC15

BESTROPHIN-3 IS IMPORTANT FOR VASOMOTION

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We have recently characterized a cGMP-dependent Ca^{2+}-activated Cl^- current ($I_{Cl,cGMP}$) with unique characteristics in smooth muscle cells (SMCs) and suggested that this could be important for vasomotion in mesenteric arteries. We hypothesized that a bestrophin could be responsible for $I_{Cl,cGMP}$ based on similarities in the membrane currents and on the distribution pattern of $I_{Cl,cGMP}$ and bestrophin-3 protein expression in different SMCs. Bestrophin-3 was downregulated with small interference (siRNA) in rat mesenteric small arteries *in vivo.* Non-related siRNA and mutated bestrophin-3 siRNA were used as the controls. Downregulation induced by specific siRNA was evident by reduced expression of bestrophin-3 mRNA and protein (to 24+/-17 % and to 58+/-9 % of the control, n=3) without an effect on other bestrophin isoforms. SMCs downregulated for bestrophin-3 demonstrated a significant reduction of $I_{Cl,cGMP}$ (by 98+/-0.2%, n=7 at -60 mV), while the "classical" Ca^{2+}-activated Cl^- current was unaffected. Surprisingly, arteries with reduced bestrophin-3 were more sensitive to noradrenaline than control vessels. The downregulation of bestrophin-3 significantly reduced the amplitude of arterial vasomotion (by 75+/-10 % n=6) without significant effect on frequency. We provide evidence that bestrophin-3 is essential for $I_{Cl,cGMP}$ and is important for generation of vasomotion in rat mesenteric small arteries. This work was supported by the Danish Research Council, the Danish Heart Foundation. The Water and Salt Research Center at the University of Aarhus is established and supported by the Danish National Research Foundation.

PTC16

REGULATION OF THE INDUCIBLE L-ARGININE-NITRIC OXIDE PATHWAY BY OXIDATIVE STRESS AND STATINS

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Oxidative stress (OS) plays a critical role in the pathogenesis of atherosclerosis potentially through interaction with nitric oxide (NO) generated by the inducible nitric oxide synthase (iNOS) pathway. Recent data indicates that iNOS-induced NO may have a beneficial role in atherosclerosis by attenuating atherosclerotic lesions, enhancing endothelial integrity, and protecting against OS-induced apoptosis and necrosis. Pro-oxidants may modulate iNOS-induced NO production through alteration of the expression and/or function of the inducible L-arginine-NO pathway. We examined the effects of hydrogen peroxide, antimycin A and diethyl maleate (DEM) on this pathway in vascular smooth muscle cells. Hydrogen peroxide had little effect on NO production or L-arginine transport while antimycin A and DEM independently caused a concentration dependent inhibition of both processes. Only DEM induced heme-oxygenase-1 expression, monitored by western blotting as a marker of OS. The effects of stations on NO synthesis and L-arginine transport in the presence and absence of OS were also investigated. Atorvastatin caused a bell shaped response on NO production, iNOS expression and L-arginine transport while simvastatin only affected NO synthesis without altering transporter activity. Pravastatin was without effect on either system. Further studies are ongoing to determine the mechanisms of actions of statins, antimycin A and DEM.

PTC17

REGULATION OF CAPILLARY GROWTH IN RAT SKELETAL MUSCLE: ARE EFFECTS OF MUSCLE OVERLOAD AND ELEVATED SHEAR STRESS ADDITIVE?

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In skeletal muscle, capillary growth occurs via abluminal sprouting and longitudinal splitting, which can be initiated by muscle overload and elevated microvascular shear stress, respectively. These morphologically distinct patterns of growth suggest that discrete sets of angiogenic mediators are activated in each situation, raising the possibility of a greater effect with multiple stimuli. In this study we combined increased shear stress by administration of the alpha-1 antagonist prazosin and muscle overload by synergist extirpation (E+P) in rats to examine whether enhanced capillary growth was achieved, compared to muscle overload alone (E). Extensor digitorum longus was collected after 4 or 14 days of intervention. Histochemical analysis showed that 14 days of E+P or E induced a ~35% increase ($P<0.05$) in capillary to fibre ratio (C:F) compared to control animals with no difference in effect between the two interventions. Analysis of muscle eNOS protein showed an increased content ($P<0.05$) after 4 days in E+P which was not present in E. There was no significant increase in muscle VEGF protein with either intervention. These data suggests that a combination of muscle overload and elevated shear stress induces a greater effect on eNOS protein than extirpation alone, but does not further enhance muscle capillary growth. The project was supported by the Danish Medical Research Council.

PTC18

AN EXPERIMENTAL MODEL TO EVALUATE PHARMACOLOGICAL EFFECTS IN THE MYOMETRIAL MICROCIRCULATION OF THE PREGNANT RAT

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Local mechanisms regulating blood flow in the pregnant myometrium are still poorly understood. Uterine microcirculation has been investigated in only a very few studies, and we did not find any in which microcirculatory responses to pharmacological agents commonly given to pregnant women were analyzed. The primary reason for the paucity of this important information lies in the significant technical difficulties in reliably assessing microcirculatory variables in the contracting, pregnant uterus, in vivo. In the present study, we developed a novel technique to evaluate microvascular responses in the uterus of close-to-term rats. Anaesthesia was induced with 60 mg/kg thiopental intraperitoneally. A U-shaped incision was made on the exteriorized uterine horn. The uterine lobe was flipped over into an organ bath then restretched to its original size. A video rate camera connected to a florescence microscope in transillumination mode was used to visualize microcirculation. By exteriorization of the myometral lobe, sufficiently appropriate quality images of the microvasculature could be obtained. Motion pictures of the microcirculation were recorded. RBC velocities were determined „off-line" using CapiScope software. To test the pharmacological capability of our model, effects of tocolytics and uterotonics were examined. It was possible to quantitate the dose-dependent effects of all these pharmacons. The present study was supported by Hungarian Health Science Committee Research Grant (registry no.: ETT 332/2006).

PTC19

DESIGN AND PRELIMINARY EVALUATION OF A COOLING/HEATING DEVICE TO ASSESS MICROVASCULAR FUNCTION IN HUMAN SKIN

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In order to investigate the skin local vasomotor response to a variation in cutaneous temperature in human, we made a new local cooling/heating device associated with a laser Doppler flowmeter. We further tested the skin response to local cooling down to 15°C and its reproducibility.

The cooling/heating probe is made of paired optic fibres and a Peltier element as a thermoelectric cooler. The direction of the heat flow depends on the polarity of the voltage supplied, allowing either heating or cooling of a single surface. We performed local cooling on the forearm of 6 healthy volunteers from 33°C to 24°C and then to 15°C, twice, and repeated the measurement 7 days later. Data are expressed as cutaneous vascular conductance (CVC) in mV/mmHg.

Local cooling decreased CVC between 33°C, 24° and 15°C ($p<0.05$ for all series, Friedman test). The reproducibility was good, with median (interquartile) CVC at 15°C of 0.43 (0.04) and 0.41 (0.11) at day 1, and 0.42 (0.46) and 0.39 (0.34) at day 7. The cooling/heating device was well tolerated by all the volunteers. This new tool could be of great interest to assess microcirculation function in human skin in diseases such as Raynaud's phenomenon.

PTC20

EFFECTS OF GHRELIN ON ISOLATED CANINE CORONARY ARTERIOLES

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Ghrelin (GHR) is a newly discovered gastrointestinal endocrine regulatory peptide with multiple functions. Its production has recently been described in various tissues including the myocardium where it may have cardioprotective effects. However, contradictory data are available on the vascular actions of GHR in different organs and species. The aim of this study was to characterize the direct effect of the peptide on canine coronary bed. Intramural coronary arteriole segments (n=7, diameter approx. 250 microm) were isolated from healthy dog hearts. The cannulated arterioles were pressurized to 50 mmHg using a servo-controlled pump system. Coronary responses to GHR (30-300 nM) were recorded by microarteriography as changes of vessel diameter with or without preconstriction evoked by thromboxane mimetic U46619 (1 microM). The results showed that GHR alone can not elicit any significant effect on vessel diameter. In the case of elevated vascular tone (mean+/-SEM: 25+/-5% decrease in outer diameter) GHR evoked significant vasoconstriction with a maximum of 34+/-11 % of the effect of U46619 ($p<0.05$). The results suggest that GHR induces tone-dependent constriction of canine coronary arterioles, indicating a potential role of the peptide in the regulation of coronary function.

PTC21

CONNEXIN45 HAS A MINOR ROLE FOR THE CONDUCTION OF VASOMOTOR RESPONSES ALONG THE SMOOTH MUSCLE IN ARTERIOLES

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Gap junctions allow the conduction of vasomotor responses and the synchronization of cellular behaviour in the microcirculation. In arterioles gap junctions are composed of four different connexins (Cx40, Cx37, Cx43, and Cx45). We have shown recently that Cx40 exhibits specific properties in the endothelium that cannot be replaced by Cx45. Here we investigated the functional role of Cx45 in mice lacking Cx45 in vascular smooth muscle (Cx45SM-KO) using a nestin-promotor controlled Cre-LoxP-System. Conducted vasomotor responses initiated by KCl (3M) and ACh (10mM) were studied in arterioles (~35 microm.) of the cremaster muscle using intravital microscopy and Cx expression by fluorescence microscopy. Weak Cx45 staining was detected in vascular smooth muscle and Cx45 expression was verified by the gene-targeted approach. KCl induced a similar vasoconstriction in wildtype and Cx45SM-KO mice at the stimulation site (-39±4% vs. -37±3%). This constriction was conducted upstream with a similar decline of the amplitude in both genotypes (900µm: wt, -12±3%; Cx45SM-KO, -14±3%). The propagation of ACh-induced vasodilations was also comparable between genotypes. In summary, lack of Cx45 in the smooth muscle layer has no functional consequences for the spread of vasomotor responses despite its expression in vascular smooth muscle cells.

PTC22

CHARACTERIZATION OF INTERCELLULAR CALCIUM WAVES ARISING DURING ARTERIAL VASOMOTION

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Vasomotion consists in cyclic arterial diameter variations induced by synchronous contractions of smooth muscle cells (SMCs) of the arterial wall. However, the arteries do not contract simultaneously on a macroscopic distance, indeed a propagation of this contraction along the artery is observed. This contraction propagation is in fact induced by an increase in the intracellular calcium concentration in the SMCs, which propagates from cell to cell generating an intercellular calcium wave. We characterized these intercellular calcium and contraction waves on rat mesenteric arterial strips stimulated with Phenylephrine. Calcium dynamics in individual SMCs was analyzing on strip where the endothelium was mechanically removed. A velocity of about 100 microm./s was measured for the intercellular calcium wave. Using a tracking algorithm, we were able to follow the contraction of the arterial strip and to measure the velocity of the contraction wave, which is, as expected, of the same order. By performing experiments on strips with intact endothelium, we saw that the speed of the contraction wave remains the same, independently of the presence or absence of endothelium. These results allow to conclude that the endothelium is not the principal pathway for the intercellular calcium wave spreading between SMCs of the arterial wall.

PTC23

GHRELIN INDUCES VASOCONSTRICTION IN THE RAT CORONARY CIRCULATION

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Ghrelin, a 28-amino-acid peptide, is the endogenous ligand for a previously identified orphan growth hormone secretagogue receptor (GHS-R). Although both ghrelin and its specific receptor are expressed in coronary arteries, the functional significance of the ghrelin/GHS-R system in the regulation of coronary vascular tone is not known. In isolated perfused rat hearts, infusion of ghrelin (0.1 to 1 nmol/L) for 60 minutes induced a dose-dependent coronary constriction. The effect was most pronounced at the concentration of 1 nmol/L, which resulted in a 44+/-9% increase in perfusion pressure ($P<0.01$). Similarly, administration of 1 nmol/L ghrelin resulted in a slow constriction of isolated coronary arterioles (12+/-2%, $P<0.05$). Furthermore, ghrelin significantly enhanced the pressure-induced myogenic tone of arterioles between 40 and 120

mmHg (P< 0.05). The ghrelin-induced vasoconstriction was blocked by diltiazem, an L-type Ca^{2+} channel blocker, and bisindolylmaleimide, a protein kinase C inhibitor in both preparations. In conclusion, we provide the first evidence that ghrelin has a coronary vasoconstrictor action that is dependent on Ca^{2+} influx through L-type Ca^{2+} channels and activation of protein kinase C. Since ghrelin binding is significantly up-regulated in atherosclerotic coronary arteries, the peptide may aggravate coronary constriction in the diseased vessels.

PTC24

IN VITRO METHODS TO INVESTIGATE THE VASOMOTOR ACTIVITY OF MICROVESSELS

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In order to reveal their role in the regulation of blood circulation it is important to investigate the vasomotor activity of isolated microvessels, such as small arteries, arterioles, small veins and venules (and lymphatics). These vessels can be isolated from most tissues or organs (skeletal muscles, heart, brain etc.) and their vasomotor function can be assessed by use of naturally occurring vasoactive agents with known mechanisms of action and use of inhibitors of specific signaling pathways. In addition, responses of vessels to hemodynamic forces, such as pressure, flow/shear stress and viscosity can also be studied. Furthermore, the role of endothelium modifying smooth muscle responses can be assessed by using intact and endothelium-denuded microvessels. There are two main types of methods: the wire myograph and the cannulated vessels. In brief, after anesthesia the organ/tissue is excised from the animal and placed in cold physiological salt solution (PSS). Using microsurgery instruments and an operating microscope a segment of a microvessel is isolated, and transferred into a vessel chamber, which is continuously perfused with PSS. In case of the first method, two micro-wires, each secured to jaws, are inserted into the vessel lumen. Internal circumference, segment length, wall tension, isometric responses of vessels are measured by a micro-positioner and an isometric transducer In the other one, both ends of the vessel are mounted on glass micropipettes connected with silicone tubing to an adjustable PSS reservoir. In this method intraluminal flow and pressure can be controlled independently and measured by electro-manometer and flow-meter. Diameter responses of vessels can be followed by videomicroscopy equipped with a microangiometer [These instrumentations are available from Danish Myo Technology Products (www.dmt.dk), Experimetria Ltd (www.experimetria.com), and Living Systems Instrumentation (www.livingsys.com)]. These methods allow elucidating the role of microvessels in determining vascular resistance and the intracellular mechanisms responsible for it, both in physiological and pathological conditions.

PTC25

INVOLVEMENT OF LOCAL RENIN ANGIOTENSIN SYSTEM (RAS) AND NAD(P)H OXIDASE IN THE ASYMMETRIC DIMETHYLARGININE (ADMA)-INDUCED SUPEROXIDE PRODUCTION AND ARTERIOLAR VASOMOTOR DYSFUNCTION

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Our previous investigation showed that in isolated microvessels ADMA, an endogenous inhibitor of nitric oxide (NO) synthase - in addition to inhibiting flow-induced dilations of arterioles - elicited superoxide production. We hypothesized that elevated level of exogenous ADMA, by activating local RAS and vascular oxidases, elicits increased superoxide production. In the presence of indomethacin, isolated arterioles from rat gracilis muscle (~ 160 microm. at 80 mmHg) were incubated with ADMA (10-4 mol/L), which elicited significant constriction (from 162±4 microm. to 143±4 microm.) and eliminated the dilations to increases in intraluminal flow (from max.: 31±2 % to 3±1 %). In the presence of ADMA, superoxide dismutase (SOD) plus catalase (CAT), the NAD(P)H oxidase inhibitor apocynin or angiotensin-converting enzyme (ACE) inhibitor quinapril restored dilations to flow. ADMA increased the production of vascular superoxide assessed by dihydroethidium (DHE) fluorescence, which was inhibited by apocynin or quinapril. Thus, we suggest that ADMA activates the local RAS, and the released angiotensin II activates NAD(P)H oxidase; then the increased level of superoxide reduces the bioavailability of NO resulting in diminished flow-induced dilation. These mechanisms may contribute to the development of microvascular dysfunction associated with elevated levels of ADMA.
Grants: AHA NE Aff. 0555897T; Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006.

PTC26

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE LOWERS BLOOD PRESSURE NOT VIA ACUTE RELEASE OF EET/EDHF BUT BY CHRONIC AUGMENTATION OF ENDOTHELIAL NITRIC OXIDE RELEASE

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Soluble epoxide hydrolase (s-EH) rapidly hydrolyses epoxyeicosatrienoic acids (EETs), which have been proposed to serve as EDHF in specific vascular beds. Inhibition of s-EH increases EETs in vascular endothelium. We postulated that acute release of EETs may mediate the blood pressure (BP) lowering effect of s-EH inhibitors. To test this hypothesis, rats were chronically infused with Ang II (200 ng/kg/day for 28 days) (Ang II rats), which increased BP by ~40 mmHg. Single oral or intravenous administration of a selective and potent s-EH inhibitor did not lower BP in Ang II rats, although completely inhibited s-EH activity and elevated EETs concentration in the blood to a level similar to that in s-EH deficient mice. In

contrast, chronic oral treatment of Ang II rats with the same inhibitor significantly lowered BP. Impaired endothelial nitric oxide (NO) production/activity is a hallmark of hypertension. Indeed, chronic Ang II infusion caused significant impairment of acetylcholine (Ach)-induced endothelium dependent relaxation in isolated aortic rings. Chronic treatment with the s-EH inhibitor completely restored Ach-induced relaxation. These data demonstrate that BP lowering effect of s-EH inhibition is not due to acute EET/EDHF release, but chronic restoration of endothelial NO production. Thus, pharmacological inhibition of s-EH may be a promising novel approach to improve endothelial function that could provide therapeutic benefit in the treatment of cardiovascular diseases.

Thursday, August 28th
Cardiac dysfunction
Poster Session - PCD

PCD1

LOW-DOSE METFORMIN THERAPY ATTENUATES LEFT VENTRICULAR DYSFUNCTION AND REDUCES MORTALITY IN A MURINE MODEL OF HEART FAILURE

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Clinical studies indicate that metformin protects against cardiovascular disease in patients with diabetes and metabolic syndrome by actions that cannot be solely attributed to its glucose-lowering effects. We sought to investigate the potential cardioprotective effects of an acute, low-dose metformin treatment (i.e. 286-fold less than the maximum anti-hyperglycemic dose) in a murine model of heart failure (HF). Mice were subjected to myocardial ischemia for 60 min followed by reperfusion. Metformin (125 µg/kg) or vehicle (saline) was administered as a single bolus (i.v.) at the time of reperfusion and then daily (intraperitoneal) for 4 wks. Administration of metformin reduced infarct size by 30% ($p<0.001$) at 24 hrs of reperfusion and significantly ($p<0.01$) improved LV function at 4 wks of reperfusion without altering blood glucose levels. At 4 wks of reperfusion, metformin therapy significantly ($p<0.05$) increased the phosphorylation status of AMPK and eNOS, increased the expression of PGC-1 , and improved mitochondrial ATP synthesis and oxygen consumption in the hearts of treated mice. In an additional group of mice subjected to a complete occlusion of the left coronary artery, metformin reduced mortality by 50% ($p<0.05$). These findings suggest that low-dose metformin therapy may have a practical clinical use for the treatment of HF. This work was supported by the National Institutes of Health (HL-060849-08 to D.J.L. and F32 DK 077380-01 to J.W.C.) and the American Diabetes Association (7-04-RA-59 to D.J.L.).

PCD2

PREVALENT ROLE OF AKT AND ERK ACTIVATION IN CARDIOPROTECTIVE EFFECT OF CA^{2+} CHANNEL- AND ß-BLOCKERS

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We studied cardioprotective as well as Akt and ERK activating effect of a Ca antagonist and a β-blocker during ischemia-reperfusion cycle in Langendorff-perfused isolated rat hearts, and compared these properties of the substances to that of a poly(ADP-ribose) polymerase (PARP) inhibitor used as a positive control throughout the experiments. Although in different extent, all substances improved recovery of creatine phosphate, ATP, intracellular pH, and reutilization of inorganic phosphate. These favorable changes were accompanied by improved recovery of heart function parameters and reduced infarct size. In addition, all studied substances decreased oxidative damage (lipid peroxidation and protein oxidation), and activated Akt, and ERK1/2. Correlation between cardioprotective and kinase activating effectivity of the compounds proved to be statistically significant. Inhibition of Akt by LY294002 and ERK1/2 by PD98059 compromised the cardioprotective effect of all the substances studied corroborating the significance found. In conclusion, we demonstrated at the first time that activation of PI-3K/Akt and ERK2 pathways significantly contributed to cardioprotective effect of a Ca^{2+} antagonist and a β-blocker. Furthermore, we found a strong correlation between cardioprotective and kinase activating potencies of the substances studied that suggests potentiality of these kinases as drug-targets in the therapy of ischemic heart disease.

PCD3

PARTICULATE MATTER INHALATION IMPAIRS CORONARY MICROVASCULAR REACTIVITY

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We have recently shown that systemic microvascular function is impaired after inhalation exposure to fine and ultrafine particulate matter (PM). Studies have shown that exposure to airborne particle pollution increases the risk of ischemic cardiovascular events. However, it remains unclear how the coronary microcirculation contributes to such insults. We tested the hypothesis that microvascular reactivity is impaired after PM exposure in rat coronary arterioles. Rats were exposed to filtered air (control), fine PM, or ultrafine PM via inhalation at concentrations relevant to ambient air pollution (4-90 g measured pulmonary deposition). Coronary arterioles were subsequently isolated and responses to flow (FID), acetylcholine (ACh), endothelin and dea-NONO-ate were assessed. Exposure to either fine or ultrafine PM significantly impaired FID, but neither group displayed an altered response to ACh. Interestingly, vascular smooth muscle nitric oxide (NO) sensitivity (dea-NONO-ate) was decreased by exposure to ultrafine PM, but not fine PM. Furthermore, ultrafine PM exposure diminished vasoconstrictor responses to endothelin, whereas fine PM exposure had no effect on endothelin reactivity. These results suggest that ultrafine PM exposure causes significantly more microvascular dysfunction than fine PM. It is probable that such disturbances in coronary microvascular function contribute to the cardiac events associated with particle pollution exposure. Support: R01-ES015022 and HEI#4730 (TRN) and R01-HL077224 (JMD).

PCD4

CARDIOPROTECTIVE EFFECT OF RESVERATROL IN PATIENTS WITH ISCHEMIC HEART DISEASE

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It is well known, that moderate alcohol - especially red wine - consumption decreases the morbidity and mortality of cardiovascular diseases. The aim of our study was to investigate the effect of a red wine polyphenol - resveratrol - without alcohol intake on cardiac function, exercise capacity, flow-mediated vasodilation (FMD), lipid, glucose and hemorheological parameters in patients with ischemic heart disease. 40 patients with verified coronary artery disease were randomized into two groups, and 10 mg resveratrol or placebo was given to the patients for 3 months. Resveratrol improved significantly both systolic (p<0.05) and diastolic (p<0,01) left ventricular function, exercise capacity (p<0,05). We could observe a significant improvement in endothelial dysfunction assessed by FMD (p<0,05), lipid parameters (p<0,05), while in the placebo-treated group we could not see any remarkable changes. Most of the examined hemorheological parameters showed a worsening tendency, furthermore the red cell deformability and collagen-induced platelet aggregation deteriorated significantly (p<0,05) in the placebo group. Resveratrol treatment prevented this worsening. Our results show that resveratrol - even without alcohol intake and beside an adequate drug therapy - can improve cardiac performance, endothelial dysfunction and serum lipid and some hemorheological parameters in patients with ischemic heart disease.

PCD5

THE PHOSPHODIESTERASE-5 INHIBITOR VARDENAFIL IMPROVES CARDIOVASCULAR DYSFUNCTION IN TYPE 1 DIABETES MELLITUS

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Patients with diabetes mellitus exhibit generalized endothelial and cardiac dysfunction with decreased NO-production and increased oxidative stress. It has been reported, that elevated $cGMP_{ic.}$ levels contribute to an effective cytoprotection against oxidative stress. We investigated whether chronic treatment with the phosphodiesterase-5-inhibitor vardenafil could improve diabetic cardiovascular dysfunction by upregulating the NOS/cGMP pathway. Diabetes was induced in rats by ip. streptozotocin-injection (60mg/kg). Vardenafil (10mg/kg/d, treatment group) or vehicle (diabetic control group) was applied orally for 8 weeks. Left ventricular pressure-volume relations were measured by using a microtip pressure-volume conductance catheter, and indexes of contractility (slope of ESPVR (Emax) and PRSW) were calculated. In organ bath experiments for isometric tension with isolated aortic rings, endothelium-dependent and -independent vasorelaxation was investigated by using acetylcholine and sodium nitroprusside (SNP). When compared to the non-diabetic controls, diabetic rats showed impaired left ventricular contractility (Emax: 1.04+/-0.07 vs. 2.23+/-0.20mmHg/microl; PRSW: 45.39+/-2.45 vs. 76.44+/-4.06mmHg/s/microl) and a significant vascular dysfunction. Treatment with vardenafil resulted in a significantly improved cardiac function (Emax: 2.03+/-0.14 vs. 1.04+/-0.07mmHg/microl; PRSW: 76.53+/-3.49 vs. 45.39+/-2.45mmHg/s/microl) and higher vasorelaxation to acetylcholine and SNP in diabetic animals. Our results demonstrate that impaired vascular cGMP-signalling contributes to the development of diabetic cardiovascular dysfunction, which can be prevented by phosphodiesterase-5-inhibition.

PCD6

MICROCIRCULATION AND ENDOTHELIAL FUNCTION IN SYSTOLIC AND DIASTOLIC HEART FAILURE PATIENTS WITH METABOLIC DISORDERS

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Purpose: To conduct comparative analysis of metabolic indices, endothelium function and microcirculation markers as well as left ventricle condition in different types of chronic heart failure (CHF) pts.
Material and methods: 118 pts aged 25-65 years old (mean age 48,8±0,3 years old), divided into two groups were examined. The 1-st group formed 49 pts (43 men and 6 women aged 48,4±1,0 years old) with systolic CHF (EF < 45%); the 2-nd group formed 69 pts (47 men and 22 women aged 49,1±0,5 years old) with diastolic CHF (E/A < 1 and IVRT > 100 ms). 30 healthy men aged 25-45 years old formed the control group. Besides standard clinical and biochemical investigation oral glucose tolerance test (GTT), insulin, C-peptide values were studied. Index HOMA was calculated, BNP-levels, cytokine system (IL-1B, IL-4, TNFa), nitric oxide (NO), C-reactive protein (CRP), and microalbuminuria (MAU) by IFA-method were studied.
Results: The disease caused CHF duration and main CHF-clinical symptoms remoteness were significally more (p < 0,001) in diastolic HF pts. At the same time in 2-nd group pts 6-minutes walking test results were better, than in 1-st group pts (290,4± 13,1 и 387,5± 4,3 meters, p <0,001 accordingly). Lipid metabolism absolute values were more normal in both groups pts, but without significant differences between groups. Insulin, C-peptide values, index HOMA were more (p<0,001) in 2-nd group pts. MAU-values which considers as general marker of microcirculatory disorders were more in diastolic CHF than in systolic CHF group pts (13,1± 0,7 and 9,6± 0,8 mg/l accordingly; p<0,001) too. There were not revealed differences between NO and CRP-values in both group pts, but their values were higher than in control group (p < 0,001). TNF-a concentration was more in 1-st group. Differences between IL-1 and IL-4 – values in 1-st and 2-nd group pts were not

revealed, but these indices were significantly more than in control group. BNP-levels were significantly higher in 1-st and 2-nd group's pts than in control group, but there were not revealed significantly differences between groups of CHF-pts.
Conclusion: The same marked disorders of lipid metabolism are revealed in systolic as well in diastolic CHF pts. In diastolic CHF pts more considerable changes of carbohydrate metabolism were exposed, that is why such pts could have burden tendency and prognosis of disease. Endothelial dysfunction and microcirculatory disorders were revealed in both types of CHF. It is connected with cytokine- system activation (elevated values of IL-1,4, and TNF-a, and compensatory elevated levels of NO). Higher levels of TNFa and BNP in 1-st group pts could suggest that systolic CHF more often distinguish burden tendency and outcomes. In general systolic as well as diastolic dysfunction are accompanying serious metabolic, endothelial and microcirculatory disorders conducting the same unfavorable prognosis. Serious approaches to treatment patients just at diastolic dysfunction stage must be provided.

Thursday, August 28th
Renal microcirculation
Poster Session - PRM

PRM1

WATER-SOLUBLE COMPONENTS OF CIGARETTE SMOKE ELICIT ENDOTHELIUM INDEPENDENT RELAXATION OF RAT RENAL ARTERIES

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Epidemiological studies have shown that cigarette smoking reduces systemic blood pressure, likely via reducing peripheral vascular resistance. It is known that the kidney contributes to the regulation of blood pressure, but the cigarette smoking-induced changes in renal vascular bed have not yet been elucidated. We hypothesized that cigarette smoke elicits dilation of renal arterial vessels. Thus we examined the acute effect of water-soluble components of cigarette smoke (wCS) on isometric tension of first order renal arteries isolated from rats. Arterial rings were preconstricted by epinephrine, norepinephrine or 60mM KCl. The function of endothelium was tested by acetylcholine-induced relaxation. Increasing doses of wCS (1-5-10%) were administered into the vessels chamber. We have found that wCS elicited a dose dependent relaxation of renal arteries (wCS1%: ~50%, wCS5%: ~80%, wCS10% ~95% relaxation). Endothelium removal abolished relaxation to acetylcholine, whereas relaxations to wCS were unaffected. Similar results were obtained with freshly obtained wCS and wCS allowed to stay in buffer solution for 4h. On the basis of these data we conclude that water soluble components of cigarette smoke elicits an endothelium independent dilation of renal arteries that are unlikely to be mediated by reactive oxygen species (such as NO, superoxide). Although further studies are necessary to elucidate the chemical nature of substances responsible for arterial relaxation, we propose that cigarette smoking may influence renal blood flow and thus regulation of systemic blood pressure by renal mechanisms. Support: Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006.

PRM2

RENAL MICROVASCULAR ENDOTHELIAL CELLS DIFFERENTIALLY RESPOND TO TREATMENT WITH AN NFKAPPAB INHIBITOR IN ACUTE INFLAMMATION

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The renal microvasculature consists of arterioles, glomerular and peritubular capillaries, and postcapillary venules. Endothelial cells in these vascular beds are actively involved in renal inflammatory disease, although the complexity of molecular control thereof remains elusive. We aimed to determine renal microvascular endothelial subset specific responses to acute inflammation and treatment with anti-inflammatory drugs *in vivo* via gene expression analysis in vascular subsets.
TNFα was systemically administered to mice, with and without i.v. pretreatment with the NFκB inhibitor Bay11-7082. Kidneys were harvested after 2 hrs, and endothelial subsets were isolated from tissues by laser microdissection. mRNA levels of multiple pro-inflammatory genes were determined by real-time RT-PCR. Bay11-7082 did not affect expression of pro-inflammatory genes in whole kidney RNA extracts. Gene expression analysis in subsets of the vasculature, however, unveiled local effects of the drug on the vascular beds. The drug inhibited TNFα induced VCAM-1 mRNA levels to 47% ($P<0.001$ vs TNFα) in arterioles, and to 24% ($P<0.001$ vs TNFα) in glomeruli. In contrast, TNFα induced ICAM-1 mRNA levels were only inhibited in arterioles to 47% ($P<0.01$ vs TNFα). Venule endothelial cells were devoid of responses. Our new research strategy creates opportunities for in-depth studies into microvascular endothelial pharmacology *in vivo.*

PRM3

CONNECTIONS BETWEEN ANEMIA AND A MARKER OF OXIDATIVE STRESS, THE URINARY ORTHO-/PARA-TYROSINE RATIO

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In the background of anemia in chronic kidney disease (CKD) and diabetes mellitus (DM), the impaired synthesis or action of erythropoietin (EPO), inflammatory cytokines and oxidative stress is hypothesized.
In a cross-sectional study, we investigated i) control subjects (CONTR); ii) patients with DM; patients with CKD and patients with DM and CKD (DM+CKD). Groups were matched for serum EPO levels. The hydroxyl free radical marker ortho-tyrosine (o-Tyr) and the physiological isoform para-tyrosine (p-Tyr) were determined in collected urine using HPLC via autofluorescence.
We found that, hemoglobin (Hb) level of the CKD and DM+CKD groups was lowest. o-/p-Tyr ratio correlated with Hb ($r=-0.346$; $p<0.001$). Correction for creatinine clearance (C_{cr}) had no influence. However, when correcting for estimated clearance markers calculated upon the Cockroft-Gault, MDRD or MCQ formulas, the correlation was not significant. In linear

regression models, o-/p-Tyr ratio was independent predictor of Hb only, if C_{cr} was used to characterize renal function in the model. However, when using MDRD or MCQ formulas, the o-/p-Tyr ratio became not a significant predictor of Hb.
Concluding, we found a connection between an oxidative stress marker and anemia, independent of serum EPO. The confounding role of renal function in the background cannot be ruled out.

PRM4
SUBPODOCYTE SPACE IN HUMAN RENAL GLOMERULI
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The urinary or Bowman's space consists of three interconnected urinary spaces forming drainage pathways for ultrafiltrate (Neal CR et al JASN 2005 16:1223, Neal CR et al Am J Physiol 2007 293:F1787). Glomerular filtrate may enter one of these restrictive spaces under the podocyte (subpodocyte space, SPS) before passing to other spaces. We have measured and reconstructed SPS in rats and observed SPS in pigs, frogs, mice and fish. Here we report on SPS in normal human glomeruli and compare with rat SPS. SPS were analysed from 4 glomeruli of normal human Kidney. Serial ultrathin sections of kidney were used to reconstruct glomerular regions into 3D models. SPS parameters were measured and compared (Mean±SEM, unpaired t-tests). The SPS height from the glomerular filtration barrier was higher in human than in Wistar rats (0.54±0.07µm, 0.24±0.08µm respectively, $p<0.05$). however the outflow through the SPS exit pore is the same in human and rat (0.25±0.08µm, 0.24±0.07µm respectively). The foot processes anchoring the podocyte to the filtration barrier in the human SPS obscure 20±3% of the SPS filtration barrier (25±3% in rat). Human SPS is similar to rat and similarly provides a further resistance to urinary outflow away from the human glomerular filtration barrier.

PRM5
MANIFESTATION OF SYSTEMIC VASCULAR DISEASE: MICROALBUMINURIA AND RENAL DYSFUNCTION IN ACUTE STROKE
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Systemic microvascular disorders can occur in various organs at different time. Previous studies have shown that renal dysfunction is associated with increased risk of ischemic stroke and suggested that patients with chronic kidney disease should be considered as a high-risk group for ischemic stroke. Thus, we hypothesized that renal dysfunction is present in acute stroke patients. In acute stroke patients (n=131), diagnosed by clinical symptoms and brain CT, serum creatinine levels was measured and the glomerular filtration rate (eGFR) was estimated. In addition, in morning spot urine specimen albumin to creatinine ratio was measured to characterize microalbuminuria. In 27% of stroke patients we have found renal dysfunction, indicated by the fact that their eGFR was below 60 ml/min/1.73m2 and 71% of patients with renal dysfunction had microalbuminuria (indicating microvascular impairment). In addition, in all the stroke patients we have found microalbuminuria in 54% and 50% of these patients had reduced eGFR. Although these studies require further clinical investigations we suggest that in most ischemic stroke patients a systemic vascular disorder is present contributing to the development of renal dysfunction. Thus the early characterization of kidney function in ischemic stroke patients seems to be important to prevent further development of renal and systemic microvascular diseases.
Grants: Hungarian Sci. Res. Funds/OTKA -T48376, K67984; Health Sci. Council/ETT 364/2006; Hungarian Neuroimaging Foundation;

PRM6
LEUKOCYTE ACTIVATION MARKERS, NITRIC OXIDE METABOLITES AND OXIDATIVE STATUS IN CHRONIC RENAL FAILURE IN CONSERVATIVE THERAPY
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Cardiovascular diseases are a principal cause of morbidity and mortality in chronic renal failure (CRF), that is considered a condition of immunological alteration involving also leukocyte function. Moreover, in CRF the balance between oxidant production and antioxidant protection is shifted towards oxidative stress. We evaluated, in 41 subjects (25 men and 16 women, mean age 64.7 +/-11.1 years) with clinically stable CRF, some parameters reflecting leukocyte activation (elastase, myeloperoxidase), plasma NO metabolites (NOx), the oxidative status (thiobarbituric acid-reactive substances (TBARS) and the total antioxidant status (TAS). We found a significant increase of elastase (92.44 +/- 31.49 ng/ml vs 59.73 +/- 17.6 ng/ml; $p < 0.001$), TBARS (10.61 ± 1.95 nmol/ml vs 5.59 +/- 1.27 nmol/ml; $p < 0.001$) and NOx (74.19 +/- 69.05 microM vs 24.38 +/- 15.67 microM; $p < 0.001$), without any significant variation of myeloperoxidase and TAS. In this group of CRF subjects, no correlation was found between the examined parameters, creatinine level, creatinine clearance, leukocyte count and hemoglobin level. This research furtherly supports the role of activated leukocytes in the development of vascular complications. The examination of leukocyte function and oxidative status could become a reliable tool to verify the clinical course in CRF.

Thursday, August 28th
Cerebral microcirculation
Poster Session – PCM

PCM1

MORPHOMETRY OF THE HUMAN CEREBRAL CORTEX MICROCIRCULATION: GENERAL CHARACTERISTICS AND SPACE-RELATED PROFILES

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Studies on human brain microcirculation have thus far yielded few quantitative data, preventing the closest possible interpretation of functional imaging methods such as fMRI and PET that necessarily rely on robustly delineated morphology of haemodynamic systems. Inadequate data in this area can lead to severe underestimation of the spatial specificity of the BOLD response. We took thick sections of Indian ink injected human brain and, using confocal laser microscopy and a novel three-dimensional computer-assisted method (Cassot et al Microcirculation 2006) we extracted and analyzed hundreds of thousands of vascular segments within a large area of cortex.
From this database the global densities, the statistical distributions of diameters and lengths were analyzed, separating the tree-like and the net-like parts of the microcirculation. Through a global heterogeneity, our analysis quantified the variations in volume density along the cortical depth and along directions parallel to the cortical surface. These results showed a high density area in the middle third of the cortex and a periodic organization along the cortical surface and were compared to recent animal fMRI studies at an intracortical level. (Lauwers et al Neuroimage 2008). These morphometric parameters are all key requirements for a sound model of cerebral microcirculation.

PCM2

TOPOLOGY AND SCALING LAWS FOR BRANCHING VESSELS OF HUMAN CEREBRAL CORTEX

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Vascular architecture, particularly of cerebral microvessels, has profound implications for both health and disease. Digital three-dimensional images of the microvascular network were obtained from thick sections of India ink-injected human brain by confocal laser microscopy covering a large zone of secondary cortex. A novel computer-assisted method was developed to extract the skeleton and measure the diameter at every vertex.
In this paper, we focused on the topology of cortical tree-like vessels that we separated from the mesh-like or capillary part of the network. Using Strahler taxonomy, the fractal nature of these branching networks was emphasized. Fractal dimension is normally distributed about 1.93 with a small variance. The variability in vascular branching data around this general ordering scheme was statistically characterized. Using stem-crown decomposition, power-scaling laws were shown to rule the relationships between integrated parameters such as the distal cumulative length or volume or the normalized flow. This led us also to an experimental justification of the allometric equation between mass and metabolic rate. Inversely, power law model did not fit adequately the relationships between local parameter such as diameter and integrated ones. As a consequence, generalized Murray's law did not appear as a valid principle for the design of cerebrovascular bifurcations.

PCM3

DILATIONS OF MIDDLE CEREBRAL ARTERIES OF SALT SENSITIVE-RATS ARE RESTORED BY RECEIVING PORTIONS OF BROWN NORWAY CHROMOSOME 13 CONTAINING THE BN RENIN ALLELE

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Middle cerebral arteries (MCA) of Dahl salt-sensitive (SS) rats exhibit an impaired relaxation in response to vasodilator stimuli, such as low PO_2 and acetylcholine (Ach), even when the rats are normotensive and fed a low salt diet. Introgression of Brown Norway (BN) chromosome 13 containing the BN allele of the renin gene into the SS genetic background ($SS.13^{BN}$ consomic rats) not only restores normal regulation of plasma renin activity and circulating ANG II levels, but also restores vasodilator responses to low PO_2 and Ach. Although $SS.13^{BN}$ rats are 98% identical to SS rats genetically, chromosome 13 contains a number of genes in addition to the renin allele that could affect vascular relaxation mechanisms. The goal of the present study was to evaluate the dilator responses of MCA to low PO_2, ACh, and the stable prostacyclin analogue iloprost in SS-derived congenic rat strains receiving smaller portions of BN chromosome 13 surrounding the renin allele. In this study congenic strains 7, 8, and 9 containing successively smaller portions of BN chromosome 13, but all carrying the BN renin allele were included. All rats were fed low salt (0.4% NaCl) diet. MCA of SS rats failed to dilate, whereas MCA of all 3 congenic strains dilated in response to low PO_2, ACh, and iloprost, supporting the hypothesis that replacement of the SS renin gene with the BN renin allele rescues vascular relaxation that is lost in SS rats.

PCM4

RESTORATION OF VASODILATOR RESPONSES IN MIDDLE CEREBRAL ARTERIES OF SS-DERIVED CONGENIC RAT STRAINS RECEIVEING PORTIONS OF BROWN NORWAY CHROMOSOME 13 CONTAINING THE BN RENIN ALLELE.

I Drenjancevic-Peric, M Durand, JH Lombard
Univ. of Osijek School of Med., Osijek Croatia and Med. College of Wisconsin, Milwaukee, WI USA.

Middle cerebral arteries (MCA) of Dahl salt-sensitive (SS) rats exhibit an impaired relaxation in response to vasodilator stimuli such as reduced PO_2 and acetylcholine, even when the rats are normotensive and fed a low salt diet. Introgression of Brown Norway (BN) chromosome 13 containing the BN allele of the renin gene into the SS genetic background ($SS.13^{BN}$ consomic rats) not only restores normal regulation of plasma renin activity and circulating ANG II levels; but also restores vasodilator responses to reduced PO_2 and acetylcholine. While $SS.13^{BN}$ rats are 98% identical to SS rats genetically, chromosome 13 contains a number of genes in addition to the renin allele that could affect vascular relaxation mechanisms. The goal of the present study was to evaluate vasodilator responses to reduced PO_2, ACh, and the stable prostacyclin analogue iloprost in SS-derived congenic rat strains receiving smaller portions of BN chromosome 13 surrounding the renin allele. In this study congenic strains 7, 8, and 9 containing successively smaller portions of BN chromosome 13, all carrying the BN renin allele were included. All rats were fed low salt (0.4% NaCl) diet. MCA of SS rats failed to dilate, while all 3 congenic strains dilated in response to reduced PO_2 (0% O_2), ACh, and iloprost, supporting the hypothesis that replacement of the SS renin gene with the BN renin allele rescues vascular relaxation that is lost in SS rats.

PCM5

PLATELET AND LEUKOCYTE ADHESION IN THE MICROVASCULATURE AT THE CEREBRAL SURFACE IMMEDIATELY AFTER SUBARACHNOIS HEMORRHAGE

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OBJECTIVE: Pathophysiology after subarachnoid hemorrhage (SAH) has not been well examined. We observed platelet-leukocyte-endothelial cell interactions as indexes of inflammatory and prothrombogenic responses in the acute phase of SAH, using an in vivo cranial window method. METHODS: Subarachnoid hemorrhage was induced in C57Bl/6J mice by the endovascular perforation method. Regional cerebral blood flow (rCBF) was measured with laser-Doppler flowmetry. The platelet-leukocyte-endothelial cell interactions were observed with an intravital microscopy 30 minutes, 2 hours, and 8 hours after SAH. The effect of P-selectin antibody and apocynin on these responses was examined, and compared with a different SAH model, in which autologous blood was injected into the foramen magnum. RESULTS: SAH was accompanied by a 60% decrease in rCBF, whereas no changes in rCBF were observed on the contralateral side. SAH elicited time- and size-dependent increases in rolling and adherent platelets and leukocytes in cerebral venules, attenuated by a P-selectin antibody, or apocynin. There was no significant blood cell recruitment in the blood-injected SAH model. CONCLUSION: SAH at the skull base induced P-selectin- and oxygen radical-mediated platelet-leukocyte-endothelial cell interactions in venules at the cerebral surface. These early inflammatory and prothrombogenic responses may cause a whole brain injury immediately after SAH.

PCM6

CEREBROVASCULAR PROTECTION BY PITUITARY ADENYLYL CYCLASE ACTIVATING POLYPEPTIDE (PACAP) AND VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AFTER GLOBAL CEREBRAL ISCHEMIA IN PIGLETS

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Impairment of cerebrovascular reactivity (CR) contributes to ischemia/reperfusion (I/R)-induced neuronal damage. The putative protective properties of PACAP and VIP against ischemic brain damage have not been investigated in the newborn. In anesthetized piglets (n= 62), we tested if PACAP isoforms (PACAP27 and 38) and VIP preserve CR to endothelium- or neuronal-dependent, I/R-sensitive vasodilator responses. Pial arteriolar diameters were determined via closed cranial window/intravital microscopy in response to hypercapnia (inhalation of 5 and 10% CO_2) which elicits endothelium dependent vasodilation or topical N-methyl-D aspartate (NMDA, 10^{-4} M) which activates the neurovascular axis, before and 1 hr after I/R. Prior to I/R, non-vasoactive doses of PACAP27, 38 (10^{-8}M, for 30 min), VIP (10^{-9}M, for 20 min) or vehicle were applied onto the cortex. I/R attenuated hypercapnia- and NMDA-induced vasodilation that was preserved both by PACAP27 and 38. For instance, postischemic CR to NMDA (vehicle vs. PACAP38; CR expressed as % of response before I/R, mean +/- SEM, n=6-8) was 30+/-12% vs. 86+/-6%, and CR to 10% CO_2 was 45+/-9% vs. 90+/-7%. VIP preserved the response to hypercapnia, but not to NMDA. In conclusion, PACAP- and VIP-induced neuroprotection is likely mediated in part by preservation of I/R-sensitive cerebrovascular mechanisms.
Supported by the OTKA (K63401 and K68976) and byETT (194042006).

PCM7

5-CARBOXAMIDOTRYPTAMINE REVEALS THE VASOCONSTRICTOR 5-HT RECEPTOR SUBTYPES OF THE RAT MIDDLE CEREBRAL ARTERY

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Exaggerated vasoconstriction is partly due to 5-HT_{1B} receptor upregulation in cerebral vessels under pathophysiological conditions (Hansen-Schwartz, et al., 2003, Beg et al., 2007), but vasoconstrictor 5-HT receptors are not characterized in the rat middle cerebral artery (MCA) under physiological conditions. The vasoconstrictor effects of various 5-HT agonists were measured without and with pre-treatment with SB 216641, a 5-$HT_{1B/1D}$ antagonist, or ritanserin, a 5-HT_{2A} antagonist, in endothelium-denuded MCAs of rats, *in vitro*. All agonists constricted the MCA. The concentration-response curve of 5-carboxamidotryptamine (5-CT) could be separated into 2 sigmoid curves (1st and 2nd phases). The order of potency of the compounds was: 5-CT (1st phase) > 5-HT > sumatriptan > 8-hydroxy-dipropylamino-tetralin (8-OH-DPAT) > 5-CT (2nd phase). The 8-OH-DPAT- and sumatriptan-induced vasoconstrictions were exclusively mediated via 5-HT_{2A} and 5-HT_{1B} receptors, respectively. SB 216641 and ritanserin antagonized the 1st and 2nd phase of the 5-CT-evoked contractions, respectively. Thus, 5-CT revealed the two vasoconstrictor 5-HT receptor subtypes in the rat MCA. Vasoconstrictions evoked by 5-HT were depressed by sb 216641 and ritanserin at low and high concentrations, respectively, suggesting greater physiological role for 5-HT_{1B} than 5-HT_{2A} receptors. In conclusion, 5-HT can regulate cerebral blood flow via vascular smooth muscle 5-HT_{1B} receptors in the rat.

PCM8

PIAL MICROVASCULAR EFFECTS OF QUERCETIN DURING TRANSIENT BILATERAL COMMON CAROTID ARTERY OCCLUSION IN RATS

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This study was aimed to assess the in vivo protective effects of quercetin on pial microvascular responses during transient bilateral common carotid artery occlusion (BCCAO). Rat pial microcirculation was observed by intravital fluorescent microscopy technique. Permeability increase, leukocyte adhesion, perfused capillary length and capillary red blood cell velocity was determined by computer-assisted methods. In control rats, BCCAO and reperfusion caused constriction of pial arterioles, leakage of fluorescent tracer, increase of leukocyte adhesion, decrease in the capillary perfusion and capillary red blood cell velocity. Quercetin (1, 2.5 and 5 mg/Kg body weight) infused before BCCAO, dose-dependently determined vasodilation and prevented microvascular damages. Inhibition of the nitric oxide synthase by L-NAME prior to quercetin blunted vasodilation, but prevented interstitial edema and leukocyte adhesion preserving capillary circulation. Inhibition of protein tyrosine kinase (Herbimycin A) before quercetin did not affect the quercetin-induced microvascular response while the inhibition of protein kinase C (Chelerythrine) prior to quercetin abolished the vasodilation and did not protect against ischemia-reperfusion injury. Moreover, guanylyl cyclase inhibitor (ODQ) prior to quercetin determined vasoconstriction but preserved the other effects. In conclusion, the protective effects of quercetin appear to be due to different pathways: nitric oxide and protein kinase C intracellular signalling.

PCM9

CHANGES IN NITRIC OXIDE SYNTHASE (NOS), CYCLOOXYGENASE-2 (COX-2), SUPEROXIDE DISMUTASE (SOD) PROTEIN LEVELS IN THE EARLY PHASE OF CHRONIC CEREBRAL HYPOPERFUSION IN RATS

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Chronic cerebral hypoperfusion, a mild ischemic condition is associated with cognitive deficits. Its experimental model is permanent occlusion of the common carotid arteries (2VO) in rats resulting in neuronal damage and microglia-activation. We purposed to detect changes in the expression of some prooxidant and antioxidant enzymes in the early phase of hypoperfusion, and to determine if the changes are affected by treatment with the potent antioxidant, alpha-tocopherol. Male Wistar rats were exposed to 2VO (n=44) or sham operation (n=37) or were not operated (naive) (n=7). The animals received i.p. injections of 100 mg/kg α-tocopherol or its solvent soybean oil or saline in equal volume after surgery. Tissue samples from hippocampus and frontal cortex were taken 1 day (n=7), 3 days (n=10), 1 week (n=38) and 2 weeks (n=33) after surgery. Western blot analysis was used to determine the expression of endothelial (e), inducible (i), neuronal (n) NOS, COX-2 and manganese SOD. Hypoperfusion increased COX-2 levels (1 & 2 weeks), decreased the expression of iNOS (2 weeks). Alpha-tocopherol treatment decreased the basal COX-2 expression and counteracted the 2VO-induced changes in iNOS and eNOS. Our results demonstrate that 2VO affects the prooxidant and antioxidant systems. These changes are sensitive to alpha-tocopherol treatment. Supported by OTKA K63401 and IN69967.

PCM10

TISSUE-TYPE PLASMINOGEN ACTIVATOR IS A REGULATOR OF MONOCYTE DIAPEDESIS THROUGH THE BRAIN ENDOTHELIAL BARRIER

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The blood-brain barrier (BBB), a selective barrier formed by specialized brain endothelial cells and dependent on the presence of intracellular tight junctions, controls the entry of circulating molecules and cells into the brain and is essential for reliable functioning. In a number of neurological diseases, a severe dysfunction of the BBB is observed which is key to monocyte infiltration into the brain. These inflammatory cells release neurotoxins, activate endogenous inflammatory processes or, in the case of macrophages, phagocytose cells or cell debris, thereby significantly contributing to the development of neurological deficits. Although little is known about the underlying mechanism, extracellular proteases may regulate inflammatory cell migration into the brain. We here describe a novel role for the extracellular protease tissue-type plasminogen activator (tPA) in neuro-inflammation and provide a potential working mechanism for this. Our results show that tPA regulates BBB opening and monocyte transmigration. Monocytes, through the activity of reactive oxygen species, induce the release of tPA by brain endothelial cells, which subsequently activates the signal transduction protein extracellular signal related kinase (ERK). Importantly, tPA initiates the breakdown of the tight junction protein occludin. Together, our results hold significant promise for treatment of chronic inflammation in the brain.

PCM11

CAPILLARY INJURY IN THE ISCHEMIC BRAIN OF HYPERLIPIDEMIC, APOLIPOPROTEIN B-100 TRANSGENIC MICE

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Apolipoprotein B-100 (apoB-100) has been implicated in hyperlipidemia that contributes to vascular disorders. Our aim was to investigate whether the expression of human apoB-100 in transgenic mice and/or a high-cholesterol diet cause cerebral microvascular lesions, and whether these conditions augment cerebral ischemia-related capillary damage. Transgenic mice that express the human apoB-100 (Tg(apoB-100), n=22) and wild-type mice (C5/B6, Wt, n=25) were supplied with standard or 2% cholesterol-enriched diet for 17-19 weeks. Cerebral ischemia was induced by unilateral common carotid artery occlusion. Ipsilateral and contralateral frontoparietal cortical samples were routinely embedded for electron microscopy. Microvascular density, lumen diameter, and the ratio of capillaries with swollen astrocytic endfeet or with endothelial microvilli were calculated. The apoB-100 expression coincided with a significant decrease in cortical microvascular density (195±7 vs 223±8 vessels/mm2) and an increased capillary lumen diameter (3.16±0.5 vs 2.88±0.6 microm). Cerebral ischemia promoted the swelling of perivascular astrocytic endfeet in the ipsilateral cortex (62.1±4.2 vs 36.5±4.0 %), and reduced the ratio of intact capillaries (32.1±5.6 vs 65.2±3.7 %). The injury was not exacerbated by hyperlipidemia. In conclusion, hyperlipidemia induced by the overexpression of human apoB-100 alters the density of the microvascular network and the diameter of capillaries, which may compromise cerebrovascular reactivity during ischemia. This work was supported by the Hungarian National Office for Research and Technology (NKTH) and by the Hungarian Science Fund (OTKA K63401).

PCM12

LASER IRRADIATED THROMBUS FORMATION AND PLATELET BEHAVIOR IN MOUSE BRAIN MICROVASCULATURE OBSERVED BY INTRAVITAL FLUORESCENCE MICROSCOPY

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Platelet behavior and thrombus formation in the microvasculature of the brain has not been fully investigated due to methodological difficulties. The purpose of this study was to observe the process of laser irradiated thrombus formation and platelet behavior in the brain microvasculature of mice using intravital fluorescence microscopy.
Methods: C57 BL/6J mice (N=21) were anesthetized with chloral hydrate; their heads were fixed with a head holder, and a cranial window was made in the parietal region. Platelets, labeled by carboxylfluorescein succinimidylester (CFSE) in vitro, were injected intravenously. Laser irradiation (1250 mA, DPSS laser 532 nm, TS-KL/S2;Sankei) was spotted for 2-4 sec on pial microvessels to induce thrombus formation. Labeled platelets and thrombus were observed continuously with a fluorescence microscope.
Results: After laser irradiation to the pial artery, thrombus formed along the vessel wall immediately, and platelets accumulated on the surface of the thrombus leading to vessel occlusion followed by spontaneous recanalization within 1-2 min. Fragmentation of thrombus was occasionally seen. Platelet adhesions to irradiated area were seen thereafter.
Conclusion: Laser irradiated thrombus formation is a promising method by which to investigate the pathophysiology of the interaction between platelets and endothelial cells.

PCM13

PEPTIDERGIC REGULATION OF DIAMETERS OF CEREBRAL RESISTANCE VESSELS IN THE NEWBORN PIGLET

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Peptidergic neurotransmitters contribute to the regulation of the diameter of the cerebral resistance vessels. The aim of the current study was 1) to characterize the vasoactive properties of pituitary adenylate cyclase activating protein (PACAP) and vasoactive intestinal peptide (VIP) and 2) to examine whether they act on common pathways in the newborn cerebral circulation.
We tested if PACAP and VIP-induced cerebral vasodilation is altered by 1) the non-selective cyclooxygenase (COX) inhibitor indomethacin, 2) selective COX-1 and COX-2 inhibitors, such as NS-398 and SC-561, respectively, 3) the NOS inhibitor N-ω-Nitro-L-arginin-methyl-ester (L-NAME), or 4) the PACAP receptor antagonists, PACAP6-27 and PACAP6-38.
Pial arteriolar diameter was measured by intravital microscopy in anaesthetized piglets (n=72) equipped with closed cranial windows. PACAP38 resulted in concentration-dependent, indomethacin-sensitive pial arteriolar diameter that was significantly reduced by SC-560 (1 mg/kg iv., 10^{-6} M PACAP38, 35+/-4% vs 0.7+/-0.5%), but was unaffected by NS-398 (1 mg/kg iv.).
VIP caused a significant, dose-dependent and repeatable pial arteriolar dilation (10^{-9}M-10^{-6}M, 9+/-2% - 65+/-5%) that was virtually abolished by indomethacin only at 10^{-9}-10^{-8}M VIP (VIP 10^{-8}M: 22+/-8% vs. -3+/-3%), was unaffected by L-NAME, and reduced by the PACAP antagonists, PACAP 6-27 and 6-38 (VIP 10^{-6}M+PACAP6-38: 66+/-6% vs. 15+/-7%, VIP 10^{-7}M+PACAP6-27: 37+/-4% vs. 25+/-4%). This study was supported by ETT 194042006 and DNT 25-11-OR126.

PCM14

OPTIMIZATION OF MEDICINAL TREATMENT OF PATIENTS WITH CEREBROVASCULAR PATHOLOGY TAKING INTO ACCOUNT THEIR INDIVIDUAL SENSIBILITY TO ANTIAGGREGANTS WITH DIFFERENT CHEMICAL STRUCTURE AND MECHANISM OF ACTION.

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Purpose of research was to estimate the efficacy of long use a wide spectrum of antiaggregants for precautions and cure patients with cerebrovascular pathology (CVP). 763 patients with varied forms of CVP were investigated. The estimate of platelets individual sensibility to influence of antiaggregants with different chemical structure and mechanism of action was done. All tests were done *in vitro* with following preparations: aspirin, cavinton, trental, sermion, dipiridamol, vasobral, and antiepileptic – carbamasepin and lamotrigil. “Cuff test” was used to estimate influence of endothelium functional potential of patients to degree of suppression of platelet aggregation by taking of antiaggregants. Homocystein level was investigated as marker of endothelium damage. It was established that all used preparations efficiently inhibited platelet aggregation in a half patients only. And these drugs had proaggregative effect of varied degree of intensity in a quarter of patients. Similar variability of platelets response to antiepileptic was marked. Antiaggregant resistance developed in the patients against a background of long taking these preparations thou initially they efficiently had suppressed platelet aggregation *in vitro*. Medicinal correction of hyperhomocysteinemia, use of combination o antiaggregants with varied mechanisms of action in common with ω-3- polyunsaturated fatty acids promoted recovery of platelet sensibility to antiaggregants. So, increase of antiaggregant efficacy can be reached by combined use of drugs with varied mechanisms of action and/or in common with course of treatment by ω-3- polyunsaturated fatty acids.

Thursday, August 28th
Varia
Poster Session – PVA

PVA1

DISCREPANCY BETWEEN SIMULTANEOUS DIGITAL SKIN MICROVASCULAR AND BRACHIAL ARTERY MACROVASCULAR POST-OCCLUSIVE HYPEREMIA IN SYSTEMIC SCLEROSIS (SSc)

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Vascular impairment is a main feature of the pathogenesis of SSc, involving the macro- and the microvasculature. Our objective was to compare and correlate simultaneously measured skin microvascular and brachial artery macrovascular post-occlusive hyperemia in patients with SSc, patients with primary Raynaud's phenomenon (PRP), and healthy volunteers. Forty-two patients with SSc, 36 patients with PRP and 33 controls were enrolled. Brachial artery flow-mediated dilation (FMD) and cutaneous post-occlusive reactive hyperemia (PORH) were simultaneously recorded after a 5-minute brachial occlusion. Local thermal hyperemia, nitroglycerin-mediated dilation (NMD), intima-media thickness (IMT) and pulse wave velocity (PWV) were also assessed.
Digital cutaneous peak PORH was altered in PRP and SSc patients compared to controls, whereas FMD was not significantly different between all groups. As a result, we observed a correlation between digital peak cutaneous vascular conductance and brachial FMD in controls (r=0.49; P=0.004) but not in patients with PRP or SSc. Thermal hyperemia was altered in patients with SSc only. Brachial NMD, IMT and PWV were not different between all groups.
We observed a loss of the correlation between brachial FMD and digital cutaneous PORH in patients with SSc and PRP. Microvascular function is impaired in SSc, whereas brachial artery endothelial function is normal.

PVA2

ABNORMAL DIGITAL NEUROVASCULAR RESPONSE TO LOCAL HEATING IN SYSTEMIC SCLEROSI

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Our aim was to investigate neurovascular dysfunction using the axon reflex-dependent hyperaemia (initial peak after skin local heating) in fingers of patients with systemic sclerosis (SSc) or primary Raynaud's phenomenon (RP).
First, ten healthy subjects were enrolled to compare axon reflex-dependent vasodilation between the finger and forearm. Then, 10 RP and 16 SSc patients participated in a similar protocol, focusing on the finger circulation. Lidocaine/prilocaine cream was applied to produce local blockade of cutaneous sensory nerves. One hour later, laser Doppler probes were heated (42°C for 30 min, and 44°C for 5 min) to achieve maximal skin blood flow. Data were expressed as percentage of maximal cutaneous vascular conductance.
In controls, we observed a blunted initial peak on the finger and the forearm blunted after topical anesthesia. In primary RP patients, digital initial peak was decreased after lidocaine/prilocaine (96.7% (33.4) versus 75.9% (29.5), P=0.02). In contrast, pre-treatment did not alter the initial peak in SSc patients.
We show an abnormal digital neurovascular response to local heating in SSc. Thermal hyperaemia could be monitored as a clinical test for neurovascular function in SSc. Further studies are required to assess its correlation to the degree of peripheral vascular involvement.

PVA3

BLOOD PRESSURE AND MICROCIRCULATION POSTNATALLY IN PREMATURE INFANTS

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In 25 preterm infants (median [95% CI]: gestational age 28 [27-29] weeks, birth weight 980 [849-1103] g) the functional vessel density (FVD) and the vessel diameter were measured with Sidestream Dark Field Imaging (SDF). The sequences were analyzed off-line by Microvision Analysis Software (MAS).
In the 48 hours postnatally FVD increased significantly (from 212 [204-218] to 224 [219-232] cm/cm^2, p 0,018) with the greatest change between the 12th und 48th hour of life (from 211 [203-220] to 226 [219-232] cm/cm^2, p 0,001). The proportion of small capillaries (diameter < 10 µm) increased from 31% [28-36] to 40% [36-42], (p 0,025). The proportion of the large vessels (diameter 20 µm) declined from 8% [7-15] to 6% [5-8], (p < 0,001). The change in percentage of the medium capillaries (diameter 10-20 µm) did not reach statistical significance (from 56% [54-59] to 54% [52-57]). Blood pressure (syst., diastol. and MAP) and FVD did not correlate (MAP: r = -0,0245, p 0,85). Catecholamines/hydrocortison infusion increased FVD significantly (210 [198-221] versus 224 [212-232] cm/cm^2, (p 0,012), but not volume (≥

10ml/kg/BW 0,9% NaCl). There are significant changes in microcirculatory parameters postnatally but without direct correlation to blood pressure changes.

PVA4

DOES CHRONIC LOCAL STRICTURE OF THE RAT SAPHENOUS VEIN RESULT IN DEVELOPMENT OF VARICOSUS CHANGES?

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Aim Venous strictures frequently develop due to pathological processes or therapeutic interventions, e.g. incomplete foam-sclerotherapy. In order to better understand the mechanism of consequences caused by chronic stricture of a vein, it is needed to analyze the biomechanical remodeling of the wall as well as the remodeling of the network properties of its tributary system. Methods A plastic clip (500 micrometer) was implanted to the proximal part of the saphenous vein of adult rats to create a stricture which may cause an increase in pressure and a decrease in flow in the distal vein branch. Four and eight weeks after clipping we performed biomechanical measurements with a pressure microangiograph using cylindrical segments of the main saphenous branch distal to the clip. Pressure-diameter curves (0-20 mmHg) of the vessel segments were registered in PSS (spontaneous myogenic tone), in maximal contraction induced by 10 μM norepinephrine, in endothelial dilatation elicited by 5.5 μM acetylcholine, and finally in Ca^{++}-free solution. The contralateral saphenous vein served as control. In addition, we examined the collateral network by making plastic casts (Batson) and by careful micropreparation of the tributary system for in vivo videomicroscopic analysis. Immuno-histology tests (Ki67, CD68 and SMA) were made to confirm angiogenesis in the surrounding tissue. Results An involution of the main vein branch was found in spontaneous tone, in maximal norepinephrine contraction, and also in the relaxed state. The newly formed small vein branches filled-up in retrograde way with the Batson plastic, and we observed paintbrush-like side-branches leading both into the fascia and to the perivascular connective tissue. In certain cases varicose-like venous dilatations were seen in the vicinity of the main branch. Conclusion Changes of venous network properties and geometry in response to chronic experimental stricture resembled to those seen in varicose disease. Our chronic clip technique seems to offer a useful model to study the mechanism of this frequent disease (Support: ETT 128/2006, OTKA TO 32019, OTKA TO 42670)

PVA5

A POTENTIAL MECHANISMS FOR IMPAIRED VASORELAXATION IN A DIETARY MODEL OF METABOLIC SYNDROME

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Poor early growth confers an increased risk of developing type 2 diabetes, hypertension, and other features of the metabolic syndrome in later life. This may result from a mismatch during early life exerting permanent effects on the structure and function of key metabolic organ systems, including the vasculature. Mice (C57BL/J) were exposed to either a high fat (HF, 45% kcal fat) or standard animal chow (C, 21% kcal fat) diet throughout pregnancy and lactation. Male offspring were then continued on the same diet as their dams or crossed over onto the alternative diet, for 10 to 12 weeks post weaning. Those fed the HF diet post weaning (HF/HF and C/HF groups) gained more weight and had a higher blood pressure than the C/C and HF/C animals. They also showed an impaired endothelium-dependent relaxation to ACh and higher basal NO production ($P<0.05$). Our preliminary data demonstrate the impact of an *in utero* and postnatal HF diet on vascular function through an impairment of NO production and/or bioavailability. It further suggests that both predisposition to disease, acquired in early life, and later lifestyle may contribute to the development of cardiovascular disease which are sustained into adulthood.

PVA6

SKELETAL MUSCLE INTERSTITIAL PROSTACYCLIN I_2 AND THROMBOXANE A_2 CONCENTRATIONS DURING TRAINING AND CONTRACTILE ACTIVITY IN PATIENTS WITH ESSENTIAL HYPERTENSION.

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An imbalance between vasodilators and vasoconstrictors in muscle may contribute to the development of high blood pressure in essential hypertension. We examined the effect of acute exercise and training on the vasoconstrictor thromboxane A_2 (TXB_2) and vasodilator prostacyclin I_2 (6-keto-$PGF_{1\alpha}$) in muscle interstitial fluid with microdialysis in non-medicated subjects with mild essential hypertension (SBP>140, DBP >90). Ten subjects performed training three times/week for 16 weeks. Training lowered ($P<0.01$) MAP of the subjects from 117±3 to 110±3 mmHg. The resting concentration of interstitial TXB_2 (n=5) decreased ($P<0.05$) from 5168±1333 to 2506±469 pg/mL. Acute exercise in the untrained state lowered ($P<0.05$) TXB_2 from 5168±1333 pg/mL at rest to 3076±908 pg/mL. Training lowered ($P<0.05$) the concentration of 6-keto-$PGF_{1\alpha}$ (n=7) at rest from 608±150 to 322±88 pg/mL. In the trained state acute exercise enhanced the concentration of 6-keto-$PGF_{1\alpha}$ from 322±88 pg/mL at rest to 599±106 pg/mL. The present study shows that a period of physical training attenuates the levels of muscle interstitial TXB_2 and 6-keto-$PGF_{1\alpha}$ in individuals with essential hypertension. Furthermore, dependent on training state skeletal muscle contraction alters the muscle interstitial levels of TXB_2 and 6-keto-$PGF_{1\alpha}$. Thus, training appears to alter the formation of important vasoactive compounds in the muscle of hypertensive subjects. Support: Danish Medical Research Council, Novo Nordisk Foundation, Lundbeck Foundation

PVA7

ANALYSIS OF FIBRINOGEN BINDING TO THE ERYTHROCYTE MEMBRANE BY FLOW CYTOMETRY

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Fibrinogen is an acute phase protein that participates in microvascular events in hemostasis, inflammation and hemorheology. Regarding this latter, plasma fibrinogen is associated with erythrocyte hyperaggregation. The interaction of fibrinogen with the erythrocyte membrane is not fully understood, with published data supporting both specific and non-specific binding mechanisms.

The objective of our study was to develop a flow cytometry methodology to analyze the interaction of fibrinogen with the erythrocyte membrane.

Human blood was used, and independent flow cytometry assays were performed with either whole blood or isolated erythrocytes. In both cases the erythrocytes were identified based on the forward and side scatter characteristics, and further checked using the erythrocyte membrane marker glycophorin A. The samples were incubated with different concentrations of AlexaFluor488-labeled fibrinogen for different timepoints. Our results show that flow cytometry reliably identifies binding of the fluorescent fibrinogen to the erythrocyte.

We are using this approach to help characterize the potential erythrocyte membrane binding site for fibrinogen. More information about this possible fibrinogen-erythrocyte binding site could lead new therapeutically approaches to address the erythrocyte hyperaggregation responsible for a significant increase of the risk of cardiovascular disorders. Supported by Fundação para a Ciência e Tecnologia: PTDC/SAU-OSM/73449/2006

PVA8

ENDOGENOUS MODULATION OF HUMAN ERYTHROCYTE NITRIC OXIDE EFFLUX

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Non-neuronal acetylcholine (ACh) triggers nitric oxide (NO) mobilization and efflux in human erythrocytes. This effect may hypothetically be associated with the degree of band-3 protein phosphorylation, the intracellular redox thiol status and adenylate cyclase (AC) or guanylate cyclase (GC) activation/inhibition. The aim of this study was to assess the dependence of NO flux on those factors, under the influence of protein tyrosine kinase (PTK) and tyrosine phosphatase (PTP) inhibitors, acetylcholinesterase substrate (ACh) and inhibitor (velnacrine maleate, VM) and a thiol-reducer agent (dithiothreitol). Venous blood samples were collected from healthy subjects and divided into aliquots containing ACh, VM, PTK/PTP inhibitors, dithiothreitol and AC/GC inhibitors. NO levels were measured in erythrocyte suspensions (haematocrit 0.05%) by an amperometric method (commercial NO sensor). Dithiothreitol decreases erythrocyte NO efflux ($p<0.0001$), an effect not reverted by ACh or VM which are NO-releasing trigger and inhibitor, correspondingly. GC inhibition significantly increases NO levels, which is further decreased by the presence of ACh and VM. Higher concentrations were additionally observed with phosphorylated band-3. Also, acetylcholinesterase effectors enhance NO efflux when band-3 is dephosphorylated ($p<0.01$). NO efflux is strongly influenced by erythrocyte band-3 phosphorylation degree, cyclases enzyme activity and thiol status activation.

PVA9

MEASUREMENT WITH MC-FAN OF THE FLUIDITY OF BLOOD AND RAW MILK FOR SIMULTANEOUS ASSESSMENT OF MILK QUALITY AND HEALTH CONDITION INCLUDING MASTITIS OF DAIRY CATTLE

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MC-FAN is an instrument developed by one of the present authors (Y.K.) to measure whole blood flow or fluidity through a microchannel array (MCA; parallel array of a large number of true-capillary size identical microchannels). Blood fluidity through MCA particularly depends on leukocyte and platelet counts and their activities, and is shown useful for health diagnosis from data so far collected on human subjects. In the present study, we show an applicability of MC-FAN, equipped with MCA defined for human blood, to the measurement of fluidity of cow blood and raw milk (pre-milking and milking), and discuss possibility of the measures to evaluate milk quality and health condition and further to detect early mastitis for individual cows. Blood passage time fell in a similar range (51.5+/-7.9 sec for n=8 in June) to that of healthy human subjects. Passage times of blood and raw milk obtained for the same group in the next month (July) were 53.4+/-10.0 sec, 21.0+/-10.1 sec, and 43.5+/-27.8 sec for blood, pre-milking, and milking, respectively.

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