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Implication of Dipeptidylpeptidase IV Activity in Human Bronchial Inflammation and in Bronchoconstriction Evaluated in Anesthetized Rabbits

B.N. Landis[a] E. Grouzmann[e] M. Monod[f] N. Busso[g] F. Petak[h]
A. Spiliopoulos[b] J.H. Robert[b] I. Szalay-Quinodoz[c] D.R. Morel[d] J.S. Lacroix[a]

[a]Rhinology-Olfactology Unit, Department of Otolaryngology, and Departments of [b]Thoracic Surgery, [c]Pathology and [d]Anesthesiology, University Hospital of Geneva, Geneva, and [e]Division de Pharmacologie et Toxicologie Cliniques, Centre Hospitalier Universitaire Vaudois, [f]Service de Dermatologie et [g]Service de Rhumatologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [h]Department of Medical Informatics and Engineering, University of Szeged, Szeged, Hungary

Key Words
Bronchial mucosa · Bronchoconstriction · Dipeptidylpeptidase IV · Histamine · Inflammation · Nasal mucosa · Upper and lower airways

Abstract
Background: Decreased dipeptidylpeptidase IV (DPPIV) activity within the human nasal mucosa has previously been shown to contribute to the severity of chronic inflammatory rhinosinusitis. ***Objective:*** To investigate and correlate the role of DPPIV activity with regard to bronchial inflammation. ***Methods:*** DPPIV/CD26 activity/concentration was investigated in the bronchial tissue of human subjects suffering from chronic bronchial inflammation. In addition, the effect of a recombinant *Aspergillus fumigatus* DPPIV (fuDPPIV) was investigated on histamine-induced bronchoconstriction in anesthetized rabbits. ***Results and Conclusions:*** DPPIV/CD26 was present in submucosal seromucous glands, in leukocytes and to a very low degree in endothelial cells of human bronchi. DPPIV activity was correlated with tissue CD26 content measured by immunoassay. As previously reported for the nasal mucosa, DPPIV/CD26 activity was inversely correlated with the degree of airway inflammation. Systemic pretreatment with recombinant fuDPPIV markedly reduced the increase in histamine-induced airway resistance in rabbits. In conclusion, DPPIV activity modulates lower airway tone by degrading unknown peptidic substrates released by histamine in response to an allergen. Contrasting with our observations in the nose, this modulation is apparently not mediated via a neurokinin (NK1) receptor.

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Introduction

Incidence and severity of chronic airway inflammation such as asthma and rhinosinusitis have increased over the last 20 years, resulting in substantial morbidity, mortality and economic costs [1]. These inflammatory conditions are essentially treated with high-potency inhaled corticosteroids, long-acting β-adrenergic bronchodilators and leukotriene modifiers. However, in spite of the progress in our understanding of asthma pathophys-

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Fax +41 61 306 12 34
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Basile N. Landis, MD
Unité de Rhinologie-Olfactologie, Service d'ORL et de Chirurgie cervico-faciale
Hôpitaux Universitaires de Genève, Rue Micheli-du-Crest 24
CH–1211 Geneva (Switzerland)
Tel. +41 22 372 82 62, Fax +41 22 372 82 40, E-Mail Basile.Landis@hcuge.ch

iology, by far not all mechanisms and factors implicated in the generation of chronic inflammatory airway diseases are yet known. Besides cholinergic vasodilatation within the bronchial and nasal mucosa, non-adrenergic, non-cholinergic or sensory vasodilator mechanisms have been described in the bronchi [2]. These mechanisms contribute to airway dysfunction and are mainly mediated by neuropeptides and cytokines [3].

Many neuropeptides have been localized to sensory nerves in the respiratory tract of various species including humans [3]. Neurogenic inflammation evoked by sensory neuropeptides could also be involved in the pathogenesis of chronic inflammation of the airway mucosa.

Recently, we demonstrated that substance P (SP), a well-known peptide that causes vasodilatation, increased capillary permeability, and recruitment of inflammatory cells contributes to the inflammatory condition of the nasal mucosa [4]. Furthermore, an enzyme implicated in the degradation of several neuropeptides, amongst them SP, is inversely correlated with the inflammatory infiltration of the nasal mucosa in patients suffering from chronic rhinosinusitis [4]. This enzyme, named dipeptidylpeptidase IV (DPPIV) or CD26 is a specific serine exopeptidase that removes Xaa-Pro dipeptides from several proinflammatory neuropeptides such as SP, as well as chemokines such as RANTES and stroma-derived factors, resulting in the loss of biological activity and/or in a change in the biological function of these peptides. However, contrasting with our previous observations in the nasal mucosa, several studies indicate that DPPIV activity is rather increased during acute inflammation and promotes chemotaxis, cell proliferation, transendothelial migration and T_H1 cytokine secretion, and decreases T_H2 phenotype [5].

Therefore, DPPIV might have a dual effect in inflammatory diseases: on the one hand, it could be beneficial for cleaving proinflammatory substrates during the acute and early phase of the inflammatory response, but it could also lead to a vicious circle during chronic inflammation through its reduced activity as observed during long-lasting rhinosinusitis. Supporting this hypothesis, a recent report on arthritis confirms our findings in the nasal mucosa, demonstrating a negatively correlated DPPIV activity with the degree of inflammation [6].

The upper and lower airways represent a continuum, and many nasal diseases influence the lower airways and vice versa [7]. Therefore, the goal of the present study was to investigate whether we could extend our previous observations in the nasal mucosa [4] to the bronchial tree, confirming the intense relationship and functional similarity between the upper and lower airway mucosa.

Materials and Methods

Human Experiments

Patients

Twenty-one patients, 11 males and 10 females, suffering from asthma, chronic bronchitis or lung carcinoma, were included in the present study (table 1). All patients had to undergo thoracic surgery for medical reasons with bronchial biopsies, and lobar, segmental or subsegmental resection. The study was approved by the ethical committee of our institution, and all patients provided informed consent for the analyses of the taken tissues.

Bronchial and Nasal Inflammation

Bronchial mucosa samples were fixed in formaldehyde and dehydrated, embedded in paraffin, stained with hematoxylin-eosin and examined under a microscope with ×40 magnification. Histological analysis included the integrity of the pseudostratified columnar epithelium, the presence or absence of edema and quantification of the number of inflammatory cells within the submucosal layer, using a scale graded from 0 to 3, where 0 indicates no inflammatory cells and 3 represents abundant inflammatory cells (for further details see Grouzmann et al. [4]). The graduation was carried out by three pathologists who were unaware of the clinical and laboratory results.

Bronchial Tissue Processing

The bronchial mucosa samples obtained under general anesthesia were immediately fixed in ice-cold acetone with 2 mM phenylmethylsulfonyl fluoride and 20 mM iodoacetamide and kept at –20°C overnight. Biopsies were embedded in glycol methacrylate resin (Polysciences, Warrington, Pa., USA) and allowed to polymerize overnight at 4°C.

Antibodies

The following monoclonal antibodies were used: CD26 (clone BA5, DAKO, Carpinteria, Calif., USA) directed against DPPIV protease, dilution 1:20; CD1a (Biogenex, San Ramon, Calif., USA) for dendritic cells, dilution 1:20; CD31 (DAKO) recognizing the adhesion molecule PECAM on endothelial cells, dilution 1:20, and the polyclonal antibody CD3 (DAKO) directed against T cells, dilution 1:20.

Immunohistochemical Staining

Serial sections, 2 mm thick, were cut using a Reichert microtome equipped with a glass knife. Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase method (DAKO) with aminoethylcarbazole as substrate. The immunohistochemical evaluation of results for DPPIV activity was performed by multiple observers blinded to the other data.

ELISA of CD26 in Human Mucosa Biopsies

A sandwich ELISA recognizing human CD26 (Bender Medsystems, Vienna, Austria) was used.

Table 1. Clinical details of the included patients

Patient No.	Age years	Gender	Pathology	Inflammation degree	DPPIV activity pmol/min/mg tissue	CD26 ng/mg
1	66	f	adenocarcinoma	2	103	3
2	26	m	asthma	1	200	2.9
3	59	m	chronic obstructive pulmonary disease	2	104	1.5
4	57	m	adenocarcinoma	1	226	2.8
5	79	m	adenocarcinoma	2	183	2.7
6	87	m	squamous cell carcinoma	2	151	3.7
7	56	f	solitary pulmonary nodule	1	378	11.3
8	54	f	undifferentiated carcinoma	1	363	9.9
9	54	m	chronic obstructive pulmonary disease	3	47	0.8
10	70	m	adenocarcinoma	2	240	–
11	72	f	chronic obstructive pulmonary disease	3	36	1.3
12	69	f	squamous cell carcinoma	2	3	0.25
13	73	f	adenocarcinoma	1	261	3.95
14	78	m	squamous cell carcinoma	3	0	6.35
15	85	f	adenocarcinoma	1	260	3.78
16	86	f	adenocarcinoma	3	19	0.25
17	81	m	non-small cell lung carcinoma	3	0	0.25
18	89	m	chronic obstructive pulmonary disease	2	134	4.1
19	56	f	chronic obstructive pulmonary disease	3	47	0.25
20	70	f	adenocarcinoma	1	157	2.4
21	75	m	squamous cell carcinoma	3	0	0.25

Bronchial tissue was harvested in patients who underwent surgery or bronchial biopsy. The pathologies are listed together with the age, gender and the respective inflammatory degree and DPPIV tissue activity and CD26 concentration.

DPPIV Activity in Human Mucosa Biopsies

DPPIV activity was determined according to Scharpé et al. [8] with the following modifications: bronchial biopsies were sonicated in the presence of 0.5 ml of 100 mM Tris-HCl (pH 8) for 2 min on ice using a Branson sonifier (output 4) and centrifuged for 10 min at 15,000 rpm in a microfuge at 4°C. The supernatant was recovered and the pellet treated with 0.5 ml of 100 mM Tris-HCl (pH 8) containing 2% Triton X-100 (Pierce). The suspension was vortexed for 1 min and centrifuged for 10 min at 15,000 rpm in a microfuge at 4°C. The supernatant was recovered and pooled with the one obtained previously and stored at –20°C. DPPIV activity was determined on 1, 2.5 and 5 µl of supernatant fluorometrically using Gly-Pro-AMC (Novabiochem, Läufelfingen, Switzerland) at 5 mM final concentration for 60 min at 37°C under agitation in an Eppendorf thermomixer in 25 µl of 100 mM Tris-HCl (pH 8). The reaction was stopped by the addition of 2.5 µl of pure acetic acid. The incubation mixture was recovered in 3.5 ml of water. A blank was obtained by incubating the substrate in the absence of the enzyme. A standard curve was determined using AMC fluorescence measurement on a Perkin Elmer LS-5 fluorometer (λ excitation: 370 nm, λ emission: 460 nm). The DPPIV activities were standardized based on wet weight tissue and specific activities expressed as picomoles of substrate converted per milligram of tissue per minute.

Animal Experiments

Recombinant DPPIV and NK1 Receptor Antagonist (L733060)

A 95-kDa *Aspergillus fumigatus* DPPIV (fuDPPIV) has previously been characterized [9]. The enzyme was expressed in the yeast *Pichia pastoris* and purified to more than 99% purity assessed by electrophoresis and gel filtration. A specific activity of 40 units/mg of protein was measured. SP (1.8 µg) coincubated with 0.016 µg DPPIV for 10 min at 37°C is degraded into SP5–11 identified by mass spectrometry; if an excess of SP was added (3.5 µg), we observed a partial digestion of the peptide into SP3–11 and SP5–11 [10]. NK1 receptor antagonist (L733060) was purchased from Novabiochem.

Rabbit Experiments in vivo

Experiments were performed on 43 New Zealand rabbits of either sex (body weight, 2.5–3.5 kg). The protocol and procedures used in the present study were approved by the local ethical review committee for animal experiments and the cantonal veterinary office.

Animal Preparation. Anesthesia was induced by intraperitoneal pentobarbital administration (25 mg/kg). Fentanyl (10 µg/kg) was used for analgesia given as an intravenous bolus once per hour. Pentobarbital injection (8 mg/kg i.p.) was repeated every hour to maintain stable anesthesia. Muscle relaxation was achieved by pancuronium (0.2 mg/kg i.v.). The rabbits were ventilated with

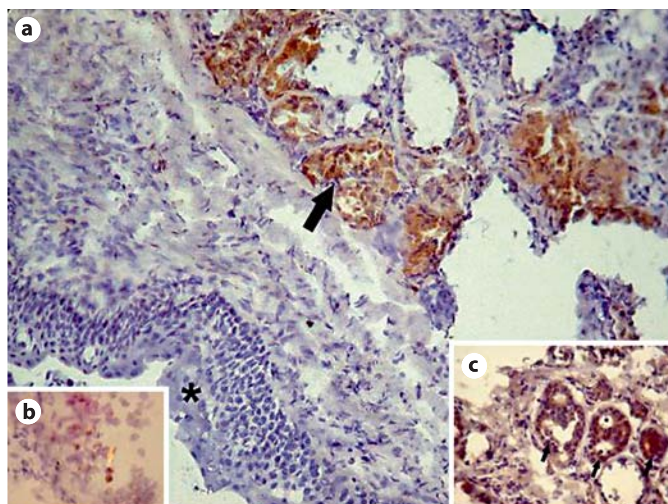


Fig. 1. Immunohistochemistry staining in human bronchial mucosa showing DPPIV-like immunoreactivity in submucosal seromucus glands (**a**, **c**, arrows). The respiratory epithelium is marked by a star. **b** shows DPPIV-like immunoreactivity in some leukocytes.

a constant-flow, small animal ventilator (Harvard Apparatus, Holliston, Mass., USA) with a tidal volume of 10 ml/kg, a respiratory rate of 25–30/min and an FiO_2 of 30%. A positive end-expiratory pressure of 5 cm H_2O was applied throughout the study. Body temperature was maintained between 37 and 38°C by a radiant heat ventilator.

The surgical procedure included a tracheotomy with a silicone catheter (3.5 mm in diameter). Another catheter was introduced into the jugular vein for drug and continuous fluid administration (NaCl 0.9%, 3 ml/kg/h). Medial sternotomy was performed with placement of a retractor to the thorax. The femoral artery was cannulated with a 28-gauge catheter (Portex, Hythe, UK) for blood sampling and continuous arterial blood pressure monitoring using a calibrated pressure transducer (model 156 PCE 06-GW2, Honeywell, Zurich, Switzerland). The rabbits were fully anticoagulated with heparin (1.5 IU/g). The preparation and stabilization of the animals lasted 90 min and the experimental protocol approximately 150 min.

Drug Administration

Bronchial drug administration was achieved by means of an ultrasonic nebulization kit (Syst'am, 2901H, Le Ledat, France). The droplet size was set at 3–4 μm and the nebulization flow at 0.75 ml/min. Histamine (2 mg/ml) was aerosolized for 5 min into the bronchial tree using a total volume of 5 ml in the nebulization chamber. The same volume of a recombinant fuDPPIV solution (50 μg/ml) was introduced in the nebulization chamber and aerosolized during 5 min.

Intravenous vascular administration was done with 1-ml syringes in which histamine (100 μg), DPPIV 2.7×10^{-2} nmol/kg (50 μg) and 1 mg of a NK1 antagonist (L-733 060) were diluted with NaCl 0.9% and DMSO, respectively.

Monitoring

Arterial blood gases were periodically checked; PaO_2 remained between 19 and 24 kPa throughout the study in all animals, indicating that the repeated histamine administration did not produce an increase in pulmonary vascular permeability potentially affecting gas exchange. At the end of each histamine challenge, a large ventilation sigh was applied in order to minimize the possible histamine-induced formation of atelectasis.

Main Outcome Measurements

Airway pressure was measured via a catheter positioned at the tip of the endotracheal tube connected to a calibrated pressure transducer (Hewlett-Packard 267B). Tidal volume was determined by integration of the respiratory flow signal measured with a heated Fleisch No. 2 pneumotachograph (Gould Godart ref. 17212) connected to a differential pressure transducer. Total lung resistance was determined by dividing the difference between inspiratory peak and end-expiratory airway pressure by inspiratory plus expiratory flow at mid-tidal volume. Data were continuously recorded using an analogue-digital converter with the AcqKnowledge software (version 3.5; Biopac Systems, Santa Barbara, Calif., USA).

Statistical Analysis

All data were computerized for statistical analysis using the GraphPad InStat3 package. Data were expressed as means ± SEM. Animal data were analyzed by analysis of variance (ANOVA) and Walloon's matched paired test, whereas human tissue findings were analyzed by rank-Spearman correlation coefficients. The significance level was set at $p < 0.05$.

Results

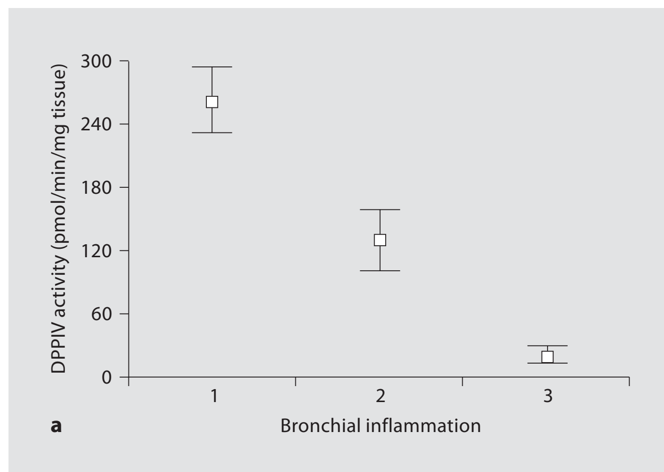
Human Data

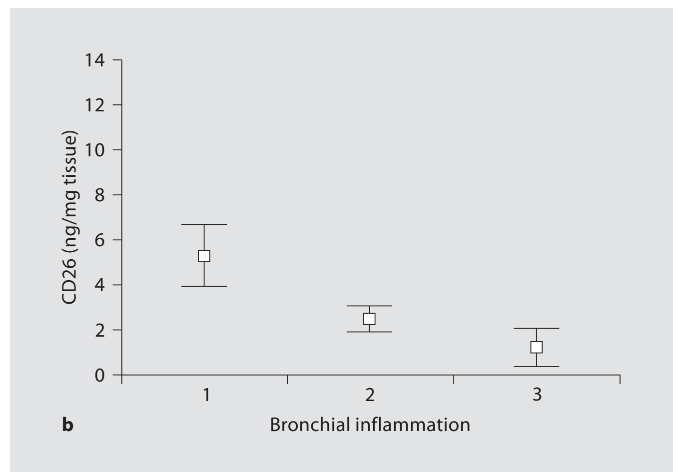
Immunohistochemistry

Immunohistochemical staining of human bronchial biopsies showed positive DPPIV-like immunoreactivity in submucosal seromucous glands (fig. 1a, c) and in some leukocytes (fig. 1b) and to a very low degree in endothelial cells. No DPPIV immunoreactivity was found in epithelial cells of the airway mucosa.

DPPIV Activity, CD26 Concentration and Bronchial Inflammation

The in vitro DPPIV activity in bronchial mucosa samples was found to be 263 ± 31 pmol/min/mg of tissue with an inflammation degree of 1; 131 ± 27 pmol/min/mg with an inflammation degree of 2, and 21 ± 8 pmol/min/mg with an inflammation degree of 3. Correlation analysis showed a significant inverse relationship between tissue inflammation and DPPIV activity in human bronchial mucosa samples ($r = -0.85$, $p < 0.001$; fig. 2a). Similarly, correlation analysis showed a significant in-





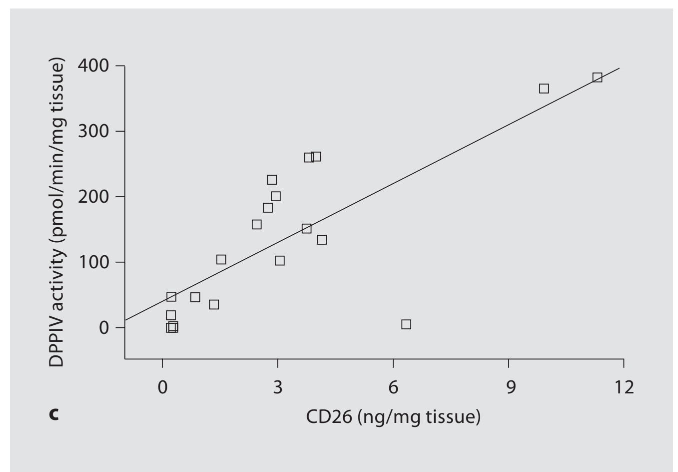


Fig. 2. a Activity within the bronchial mucosa of DPPIV activity correlates with the amount of inflammation found in the same mucosa. A significant inverse correlation was observed ($r = –0.85$; $p < 0.001$). **b** Concentration of CD26 within the bronchial mucosa correlates with the amount of inflammation found in the same mucosa. A significant inverse correlation was observed ($r = –0.56$; $p < 0.05$). **c** Significant correlation between DPPIV activity and CD26 concentration within the bronchial mucosa samples ($r = 0.77$; $p < 0.001$).

verse relationship between tissue inflammation and CD26 concentration in bronchial mucosa samples ($r = –0.56$, $p < 0.05$, fig. 2b). CD26 concentration within the bronchial mucosa was found to be 5.3 ± 1.4 ng/mg of tissue with an inflammation degree of 1; 2.5 ± 0.6 ng/mg with an inflammation degree of 2, and 1.3 ± 0.8 ng/mg with an inflammation degree of 3.

Measurements of DPPIV activity and CD26 concentration in 21 human bronchi revealed significant correlations between both of them ($r = 0.77$, $p < 0.001$, fig. 2c; table 1), indicating that the enzyme activity measured in the biopsies was essentially CD26.

Animal Data

Effect of Aerosolized Histamine on Lung Resistance

Mean lung airway resistance was determined during 1 min immediately before histamine nebulization. Histamine was then nebulized during 2 min, a sigh applied, and after 1 min, lung resistance was averaged over the following minute.

Repeated Histamine Challenge. Mean lung resistance increased from 0.179 ± 0.007 H_2O × min/ml before the first histamine challenge to 0.284 ± 0.072 H_2O × min/ml after histamine (+52.4 ± 31.1%; fig. 3a). Lung resistance was 0.188 ± 0.007 H_2O × min/ml before the second histamine challenge and rose to 0.2998 ± 0.048 H_2O × min/ml after histamine (+54.9 ± 18.8%; fig. 3a). The increase in lung resistance was not significantly different between two consecutive histamine aerosol applications ($p = 0.3$; fig. 3a).

Effect of Histamine after Aerosolized fuDPPIV Pretreatment. Lung resistance increased from 0.184 ± 0.008 to 0.343 ± 0.077 H_2O × min/ml after control histamine hallenge (+78.9 ± 31.8%; fig. 3b). Following fuDPPIV pretreatment (50 µg/ml) for 5 min, the second histamine challenge produced a significantly reduced (by 63%) in-

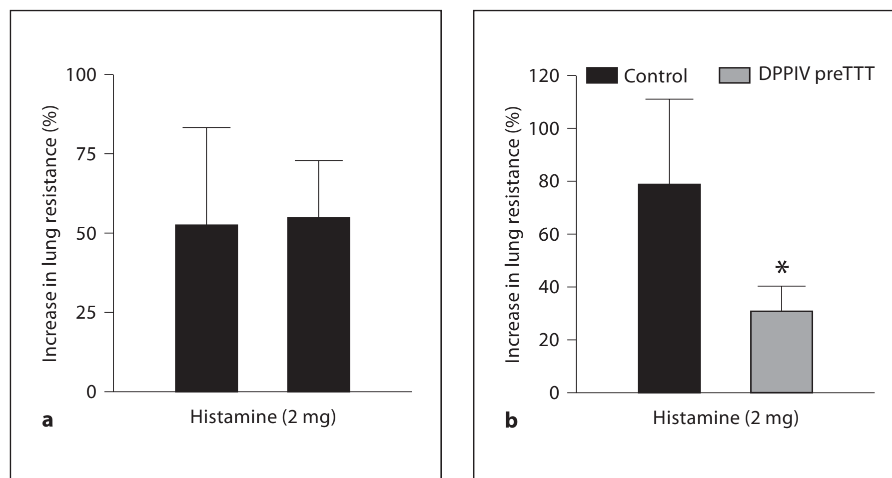


Fig. 3. **a** Percentage changes in mean lung airway resistance induced in anaesthetised rabbits (n = 12) by repeated aerosolized histamine (2 mg/ml) aerosol challenges induced an increase in lung resistance of more than 50%. The repeated stimulations were not significantly different (p = 0.3), indicating no tachyphylaxis to bronchial histamine challenge. **b** Percentage changes in mean lung resistance induced in anaesthetised rabbits (n = 13) by repeated aerosolized histamine (2 mg/ml) before and after pretreatment with aerosolized DPPIV. The pretreated airways showed a significant lower bronchoconstriction to histamine challenge (* p = 0.039).

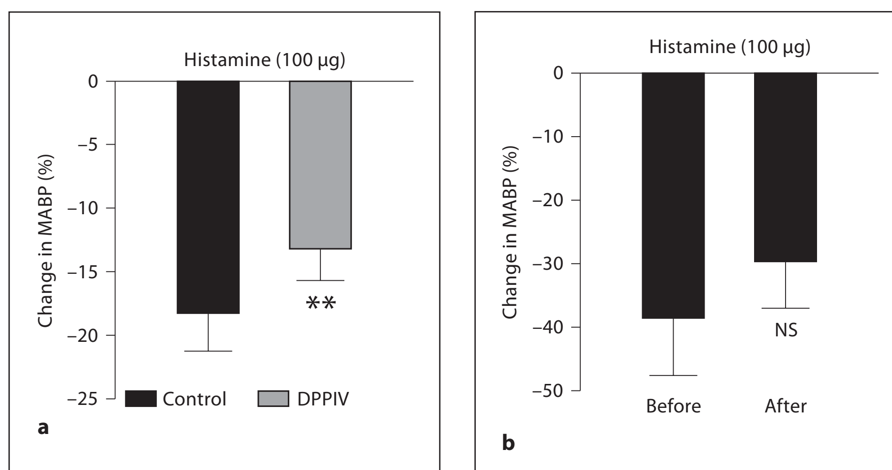


Fig. 4. **a** Percentage changes in mean arterial blood pressure after repeated i.v. histamine (100 µg) challenge (n = 10) before and after an i.v. DPPIV (2.7×10^{-2} nmol/kg) pretreatment. The pretreated airways showed a significant lower vasodilatation to histamine challenge (** p = 0.009). **b** Percentage changes in mean lung arterial pressure induced in anaesthetized rabbits (n = 6) by repeated i.v. histamine (100 µg) applications before and after NK1 antagonist L-733 060 (1 mg) i.v. given. The pretreated airways showed no significant changes in bronchoconstriction to histamine challenge (p = 0.62).

crease in airway resistance, from 0.204 ± 0.014 to 0.266 ± 0.032 $H_2O \times$ min/ml (+29.7 ± 10%; p = 0.046; fig. 3b).

Effect of Intravenous Histamine on Arterial Pressure

Effect of Repeated Intravenous Histamine. Mean arterial blood pressure (MABP) decreased by 24.5 ± 3.7% following 100 µg histamine. A second intravenous histamine injection reduced MABP by 20.6 ± 2.4% (p = 0.12 vs. the first injection).

Effect of Histamine after Intravenous fuDPPIV Pretreatment. Before pretreatment, control histamine (100 µg) injection reduced MABP by 18.3 ± 2.6%. Following pretreatment with fuDPPIV (50 µg i.v., 2.7×10^{-2} nmol/kg), a second histamine administration decreased MABP by 13.2 ± 2.4%, an effect that was significantly (p = 0.009) attenuated compared to control challenge (fig. 4a).

Effect of Histamine after Intravenous NK1 Antagonist Pretreatment. The NK1 antagonist L-733 060 did not significantly attenuate the histamine-induced hypotension observed in control conditions (NK1, 29.7 ± 7.4%; control, 38.7 ± 8.6%; p = 0.62; fig. 4b).

Effect of Intravenous Histamine on Lung Resistance

Effect of Repeated Intravenous Histamine. Lung airway resistance increased similarly during the first and second intravenous histamine injections (+52.6 ± 21 and +50.6 ± 21%; p = 0.32).

Effect of Intravenous Histamine after Intravenous fuDPPIV Pretreatment. Intravenous fuDPPIV pretreatment (50 µg, 2.7×10^{-2} nmol/kg) significantly (p = 0.002) reduced the bronchoconstriction elicited by intravenous histamine (fig. 5a).

Effect of Histamine after Intravenous NK1 Antagonist Pretreatment. Lung airway resistance increased by 42.7

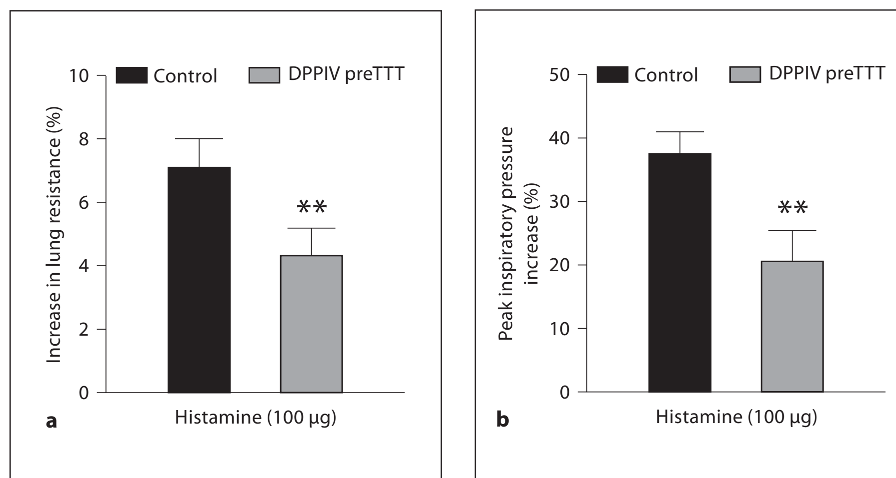


Fig. 5. a Percentage changes in mean lung resistance induced in anaesthetised rabbits (n = 5) by repeated i.v. histamine (100 μg) applications before and after i.v. DPPIV pretreatment (50 μg). The pretreated airways showed a significant lower bronchoconstriction to histamine challenge (** p = 0.002). **b** Percentage changes in mean lung resistance induced in anaesthetised rabbits (n = 9) by repeated i.v. histamine (100 μg) applications before and after DPPIV aerosol pretreatment. The pretreated airways showed a significant lower bronchoconstriction to histamine challenge (** p = 0.01).

± 19.6% after 100 μg i.v. histamine. The second histamine application after pretreatment with 1 mg i.v. NK1 antagonist (L-733 060) elicited a diminished bronchoconstriction (13.4 ± 4.9%), but that did not reach statistical significance (p = 0.162; n = 5).

Effect of Intravenous Histamine on Lung Resistance after Aerosolized Pretreatment with fuDPPIV

The average increase in lung resistance to the first intravenous histamine challenge was 37.5 ± 3.4%. The second intravenous histamine application after aerosol pretreatment with fuDPPIV elicited a bronchoconstriction of 20.4 ± 5.1% that was significantly (p = 0.01) reduced compared to the bronchoconstriction induced by intravenous histamine alone (fig. 5b).

Discussion

The results of the present study indicate that DPPIV activity is one of the enzymes involved in the regulation of inflammatory processes that occur in lower airways. First, we found that DPPIV activity was inversely correlated to the density of inflammatory cells in human bronchial mucosa, and second, we demonstrate that administration of recombinant fuDPPIV decreased the in vivo histamine-evoked bronchoconstriction and vasodilations elicited in anesthetised rabbits.

In biopsies harvested from human bronchi, DPPIV activity was correlated with CD26 concentrations, indicating that CD26 is most probably the main contributor to this activity. Interestingly, biopsies from patients with pathologies associated with tobacco consumption, e.g. squamous cell carcinoma and chronic obstructive pulmonary disease, tended to show high inflammation scores and low DPPIV activity (table 1), confirming that smoking contributes to chronic tissue inflammation. These results are in line with our previous study [4], which showed a significant correlation between nasal symptoms, low DPPIV activity as well as low CD26 concentrations in nasal biopsies from patients suffering from chronic rhinosinusitis. Although direct determination of tissue degradation products of SP resulting from DPPIV activity has not yet been reported, there are considerable arguments favoring this vasoactive peptide to be implicated in the generation of various symptoms of chronic rhinosinusitis [4].

During the last years, there has been growing evidence that nasal and pulmonary pathologies influence the natural course of each other [7]. This led to the concept of the so-called 'united airways' with morphological, but also functional similarities between upper and lower airways [7]. However, SP is probably not the proinflammatory mediator involved in the protective effect of recombinant fuDPPIV since, in the present study, the NK1 antagonist was unable to attenuate the histamine-induced bronchoconstriction and vasodilatation contrary to what was reported in nasal airways [4]. The link between the degree of inflammation and DPPIV activity in tissues was not only restricted to the airways since we found also an inverse relationship between inflammatory cellular infiltration and DPPIV activity both in human arthritis and in its animal model [6]. Of note, in the case of arthritis, the substrate (or at least one of the substrates) for DPPIV was identified to be stroma-derived factor, a chemokine that loses its biological activity when degraded

by DPPIV. Thus, it appears that depending on the tissue studied (nasal mucosa, bronchi or cartilage), a similar mechanism of downregulation of DPPIV occurs during chronic inflammation involving one or several specific substrates known to play a role in the long-term pathophysiology of these diseases. However, this paradigm must be related to clinical facts, since glucocorticoids have been shown to downregulate DPPIV activity in the skin [11], whereas glucocorticoids are an efficient and well-established treatment in asthma [12]. The mechanism that therefore associates inflammation and DPPIV activity might be more complex than previously postulated [4]. Consequently, it has to be speculated to which extent new anti-diabetic drugs like vildagliptin and sitagliptin, which act trough DPPIV inhibition, will elicit side effects such as rhinitis, bronchitis, cough, angio-edema or arthritis. Our studies confirm that DPPIV/CD26 concentrations are decreased during chronic inflammation of the upper and lower airways. Therefore, a long-term treatment with DPPIV inhibitors (gliptins) may cause nasal rhinosinusitis and bronchospasm in patients predisposed to these pathologies [13, 14].

According to our initial hypothesis based on observations in the nasal mucosa [4] DPPIV was supposed to decrease the amount of sensory neuropeptides such as SP, gastrin-releasing peptide and calcitonin gene-related peptide. SP and neurokinin A are present in sensory nerves of the airway mucosa [3] and are considered to be involved in local axon reflexes [15]. Various stimuli, e.g. bradykinin, viral infection and cigarette smoke, can activate airway sensory nerves and their neuropeptide release. The biological effects of sensory neuropeptides include smooth muscle cell contraction, mucus secretion, vasodilatation, increased vascular permeability, and recruitment and activation of inflammatory cells [16]. These inflammatory defensive reactions generated by sensory nerve stimulation are known as neurogenic inflammation. Sensory neuropeptides could be implicated in the pathophysiological mechanisms of asthma since asthmatic airways are more responsive to sensory neuropeptides. In addition, increased SP-like immunoreactivity is found in mucosal biopsy specimens from asthmatic patients [17] as well as in bronchoalveolar lavage fluids and serum during acute asthma [17]. Histamine is considered a potent liberator of SP, calcitonin gene-related peptide, bradykinin or gastrin-releasing peptide, which are believed to contribute to some extent to the inflammatory response in the upper and lower airways [18, 19]. Since DPPIV has been shown to cleave most of these second mediators [20], we further investigated the in vivo potential of recombinant fuDPPIV on a histamine-induced bronchoconstriction and vasodilatation in anesthetised rabbits. Histamine was applied intravenously, causing bronchoconstriction and vasodilatation. Furthermore, histamine was also aerosolized to induce bronchoconstriction. The main increase in lung airway resistance is primarily due to the direct action of histamine on smooth muscle cells. However, histamine is probably also triggering the release of other proinflammatory mediators [20], which further increase lung resistance through vasodilation and fluid extravasation with consecutive tissue edema within the bronchial mucosa [20]. Repeated measurements revealed no tachyphylaxis to histamine. The fuDPPIV pretreatment was also tested by aerosol as well as intravenously. Interestingly, DPPIV pretreatment was able to slightly, but significantly, reduce the histamine-induced increase in lung resistance regardless of its application route. Although the present data suggest an effect of DPPIV on histamine-induced bronchoconstriction and vasodilatation, the underlying mechanism seems to be different from that observed previously in the nasal mucosa.

Histological analysis revealed DPPIV activity in submucosal glands, leukocytes and endothelial cells. Recent studies on the distribution of airway peptidases within the nasal airways showed an important aminopeptidase activity in submucosal glands [21]. In line with these observations, we found a high DPPIV-like immunoreactivity within the bronchial submucosal glands, suggesting that submucosal gland secretion plays a key role in the nasal and probably also in the bronchial mechanisms affecting the degradation of proinflammatory neuropeptides and cytokines.

In conclusion, DPPIV activity is downregulated in chronic bronchial inflammation. We postulate that DPPIV is an important component of body defense against proinflammatory agents during various chronic inflammatory diseases. The present results extend our previous reports on rhinitis and arthritis to the lower inflamed airway tract.

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