The Enterocyte as an Energy Flow Sensor in the Control of Eating

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Abstract
Fuel monitoring in the liver or hepatic portal area was historically implicated in the control of eating. According to this view, a common denominator of nutrient metabolism such as the intracellular ATP/ADP ratio was supposed to modulate eating through changes in hepatic vagal afferent signaling. More recently, this hypothesis has been questioned because hepatic parenchymal vagal afferent innervation is scarce and because experimentally induced changes in hepatic fatty acid oxidation often failed to produce changes in eating. Accumulating evidence suggests that small intestinal enterocytes rather than hepatocytes may serve as energy flow sensors in the control of eating. These recent developments are discussed here and an outline is given of the challenges of this promising new concept.

By providing fuels and essential nutrients, eating is part of the vital regulatory feedback loops that maintain homeostasis. This homeostatic function demands that eating be controlled by metabolic feedback, but the underlying mechanisms are still unclear. Attention has focused mainly on carbohydrates and fats because changes in their utilization appear to influence eating and because they are more important fuels than proteins. As discussed in other chapters in this volume [1, 2], glucose-sensing neurons in the brain are involved in glucose homeostasis and, presumably, eating control. Hypothalamic neuronal fat metabolism also affects eating [2, 3]. Neuronal energy flow supposedly controls eating through coordinate changes in the activity of AMP-kinase (AMPK) [4, 5] and the mammalian target of rapamycin (mTOR) [6], two fuel-sensitive kinases that integrate metabolic and endocrine signals.

The existence of brain fuel sensors and their homeostatic function raises the question of whether there is a role for peripheral fuel sensing in the regulation of energy balance. Several other homeostatic feedback loops include both peripheral and central monitoring of the regulated parameter and integration of all information by the higher brain centers that control the autonomic and behavioral output [7].
hierarchical organization provides backup, increases efficiency of the regulation and, hence, minimizes fluctuations of the regulated parameter. The control of eating presumably also uses peripheral monitoring of energy flow. Where could this occur?

Does the Liver Monitor Energy Flow?

Hepatic glucose monitoring was historically implicated in the control of eating [1]. Consistent with this, glucose inhibits eating more potently after hepatic portal vein (HPV) than after jugular vein administration [8]. The glucose sensors involved are likely located in the wall of the HPV [1] rather than in the liver parenchyma. Concepts of a more general energy flow monitoring function of the liver in the control of eating (ATP/ADP ratio, e.g. [9], fatty acid oxidation, e.g. [10]) proposed that hepatocyte metabolism influences eating through changes in ATP/ADP ratio, hepatocyte membrane potential and hepatic vagal afferent activity [9, 11]. One problem with this view is that there are barely any vagal afferent fibers in the liver parenchyma [12]. It is not clear, however, how many vagal afferents are required to relay a signal, because hepatocytes are electrochemically coupled through numerous gap junctions [13]. Nevertheless, several other problems remain: (1) An electrochemically encoded metabolic signal from hepatocytes would require a consistent relation between changes in hepatocyte membrane potential, vagal afferent activity, and food intake. Such a consistent relation does not exist [14]. For instance, the fatty acid oxidation inhibitor mercaptoacetate (MA) and the fructose polymer 2,5-anhydro-mannitol (2,5-AM), which decreases hepatic ATP [15], increased both food intake and hepatic vagal branch multiunit activity [16], but MA decreased hepatocyte membrane potential [17], whereas 2,5-AM increased it [18]. (2) The interpretations of many behavioral denervation and electrophysiological data were based on the assumption that the common hepatic branch of the vagus innervates primarily the liver, which is not true. Rather, the common hepatic branch carries mainly afferent fibers from the duodenum [12, 19]. Therefore, neither behavioral phenomena observed after section of this branch of the vagus [14, 20] nor electrophysiological data obtained in multiunit recordings from it [16, 21] can be exclusively linked to the liver. (3) Several dissociations between an inhibition of hepatic fatty acid oxidation by MA and stimulation of eating [7] question the hypothesis that changes in hepatic fatty acid oxidation modulate eating. (4) The lack of a differential eating-stimulatory effect of MA after HPV or vena cava infusion [22] also questions this hypothesis because such differences helped to identify the hepatic portal area as the site of action for glucose [8], glucagon [23], CCK-33, one of the forms of endogenous endocrine CCK [24], and 2,5-AM [25]. (5) Similar to glucose, glucagon and CCK-33 may inhibit eating by a direct effect on their receptors that are expressed on HPV vagal afferents. As 2,5-AM also stimulated eating after intracerebroventricular infusion or microinjection into the ventromedial hypothalamus [26], it can directly affect neurons and might therefore also act directly on common hepatic branch vagal afferents after peripheral
administration. Thus, the observed associations between changes in hepatic energy status and changes in eating [15] need not reflect causality, and the evidence for an energy flow monitoring function of hepatocytes remains weak.

**Does the Intestine Monitor Energy Flow?**

**Enterocyte Metabolism**

The eating-stimulatory effect of MA does not appear to originate in the liver but does depend on intact abdominal vagal afferents [7, 27]. This suggests that the intestine is involved. Enterocytes require large amounts of energy for nutrient absorption and have several unique features: (1) they are exposed to and utilize fuels that enter the cells from the lumen as well as from the blood; (2) independent of its origin (gut lumen or blood), glutamine is the most important fuel of enterocytes [28], accounting for about 77 and 35% of CO₂ production in the fasted and fed states, respectively. The activity of the enzyme glutaminase, which converts glutamine to glutamate, is higher in the intestine than in most other tissues. The main end product of intestinal glutamine metabolism is alanine, which enters the HPV [28, 29]. Glutamine also provides nitrogen for the synthesis of nucleotides and other N-compounds, stimulates the mitogen-activated protein kinase pathway and promotes cell proliferation in enterocytes [30].

Enterocytes also have a high capacity for glucose utilization. Hexokinase activity is high in the fed state and decreases during fasting [29]. Even when luminal glucose is available, however, complete glucose oxidation by enterocytes is low [31, 32]. A major purpose of enterocyte glycolysis is to generate C-3 compounds such as pyruvate, lactate and alanine for the liver.

Finally, enterocytes oxidize fatty acids [31, 33, 34]. Although this is of limited energetic importance in most conditions, the capacity of enterocytes to oxidize fatty acids is implicated in the adaptation to increases in dietary fat [35, 36] and in the propensity to become obese on high-fat diets [37]. Thus, while enterocytes prefer glutamine as metabolic fuel, they can metabolize glucose and fatty acids. So far, however, effects on eating of modulations of enterocyte nutrient utilization or energy status have not been studied.

We recently observed that MA acutely and potently stimulated eating after intrajejunal infusion, and that this effect required abdominal vagal afferent signaling (table 1) [Egle et al., unpubl.], as does the effect of MA after intraperitoneal injection [27]. Intrajejunal MA infusions were effective at doses that in our hands failed to stimulate eating when infused into the HPV [22], which suggests an intestinal site of action. We obtained similar results with intragastric infusion of the glucose antimetabolite 2-deoxy-d-glucose (2DG, table 1) [Schober et al., unpubl.]. Although 2DG is scarcely absorbed, these data support the idea that intestinal fuel monitoring is not limited to fatty acid oxidation.
Intestinal Vagal Afferents

Berthoud et al. [38] identified three types of vagal afferent terminal structures in the intestinal wall: intramuscular arrays (IMAs), intraganglionic laminar endings (IGLEs), and mucosal terminals without specific structures that ended freely in the lamina propria of the mucosa near the tip of a duodenal villus. Whereas IGLEs and IMAs presumably function as mechanosensors, the mucosal terminals respond to chemical stimuli, such as serotonin, glutamate, ATP, and other substances supposedly released mainly from the enteroendocrine cells.

Given the anatomical distribution of the common hepatic branch of the vagus [12, 39], it is possible that both the increase in multiunit vagal afferent activity by MA [21] and the loss of the eating-stimulatory effect of MA after common hepatic branch vagotomy [40], which were interpreted as evidence for an hepatic action of MA, resulted from an effect of MA on duodenal vagal afferents. Using multiunit recordings from both the common hepatic and the celiac branch of the vagus, Randich et al. [41] observed that HPV infusion of 800 μmol/kg MA increased the activity of both vagal branches similarly. Note, however, that the dose of 800 μmol/kg MA used in this study is higher than the threshold doses for stimulation of eating after intraperitoneal administration. We recently observed that 200 μmol/kg MA reliably increased the activity of serotonin-sensitive celiac vagal afferent single units [42] when infused into the superior mesenteric artery, which supplies a major part of the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>60 min food intake, g</th>
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<tbody>
<tr>
<td></td>
<td>Sham (n = 12)</td>
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<tr>
<td>Control</td>
<td>0.4±0.2</td>
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<tr>
<td>MA (200 μmol/kg BW)</td>
<td>1.6±0.4*</td>
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<tr>
<td></td>
<td>Sham (n = 13)</td>
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<tr>
<td>Control</td>
<td>0.3±0.2</td>
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<tr>
<td>2DG (400 mg/kg BW)</td>
<td>1.9±0.4*</td>
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Separate groups of adult male rats were kept on a fat-enriched diet [10] (rats that received MA) or on ground chow (rats that received 2DG) and underwent subdiaphragmatic vagal deafferentation (SDA) or sham surgery. After recovery from surgery, infusions of MA (2.66 ml/kg BW), 2DG (2 ml/kg BW), or equivalent volumes of equiosmotic saline were given over 30 s at 3 h into the light phase in separate within-subjects crossover designs for each infusate and surgical group. The difference between treatments is greater in sham-operated than in SDA rats, *p < 0.05, sequentially rejective Bonferroni test after significant ANOVA.

Table 1. Intrajejunal mercaptoacetate (MA) and intragastric 2DG infusions acutely stimulate eating in Sham but not in SDA rats

Intestinal Vagal Afferents
small intestine. This result implicates the increase in celiac vagal afferent signaling in the eating-stimulatory effect of MA. A direct effect of MA on vagal afferent fibers can presently not be excluded, but appears unlikely because peripheral nerves have only a minimal capacity to oxidize fatty acids [21]. The data are therefore consistent with the idea that an intestinal fatty acid oxidation sensing mechanism influences eating. Further support for this idea is derived from the putative role of intestinal fatty acid oxidation in the resistance to high-fat diet-induced obesity (see above) and from the demonstration that sectioning the gastroduodenal branch of the vagus antagonized the streptozotocin-induced increase in lard intake in insulin-dependent diabetic rats [43].

**Challenges**

The idea that enterocytes serve as energy flow sensors in the control of eating raises several questions: First and foremost, how can changes in enterocyte metabolism affect vagal afferent activity? Intestinal vagal afferents terminate in the lamina propria of the mucosa, which is close to, but still separated from, enterocytes by a basal membrane [38]. This suggests that a chemical mediator released by enterocytes into the interstitial fluid in relation to intracellular energy flow modulates vagal afferent activity. Several substances could serve this function: (1) Oleoylethanolamide (OEA), the amide of ethanolamine and oleic acid, is an endogenous lipid that is synthesized by enterocytes in response to fat intake [44]. OEA inhibits eating supposedly through vagal afferent signaling [44]. (2) The volatile neurotransmitter nitric oxide (NO), which is produced in large amounts by enterocytes and may act as an eating-stimulatory signal [45]; inhibition of NO production is implicated in the vagally mediated eating-inhibitory effect of OEA [44]. (3) The excitatory neurotransmitter glutamate, which is the major derivative of glutamine, the major fuel of enterocytes. The ionotropic glutamate receptor N-methyl-D-aspartate (NMDA) is present on vagal and non-vagal intestinal afferents [46]. Glutamate increases multunit vagal afferent activity [47], and the delayed satiation in response to the noncompetitive NMDA-receptor blocker MK-801 depended on vagal afferents [48]. Also, intraperitoneal administration of MK-801 initially blocked, and later enhanced, the eating-stimulatory effect of MA [49], suggesting that NMDA receptor activation is involved in the initial stimulation of eating by MA. Of course, OEA, NO and glutamate are only examples for possible paracrine mediators, and whether the release of any of these three substances is modulated by changes in enterocyte energy flow is unknown. OEA is linked to fat intake and therefore seems unlikely to encode enterocyte energy flow, but NO and glutamate might qualify for such a broad signaling function. Further studies should address this possibility.

The hypothesis that MA stimulates eating by increasing intestinal vagal afferent activity is difficult to reconcile with the activation of vagal afferents by CCK or gastric
distension, both of which are associated with an inhibition of eating. It is unlikely that the opposite behavioral effects of CCK and MA are mediated by stimulation of separate fibers because we have observed that most celiac single units react to both MA and CCK [42]. Therefore, the different pattern of activation that MA and CCK induce, i.e., the fast, transient response to CCK vs. the delayed, long-term response to MA may encode opposite behavior, and/or the behavioral reaction (start or stop eating) to the afferent signal might depend on its integration with other, context-specific inputs (intra-meal signals for CCK vs. between meal signals for MA) in the nucleus tractus solitarii or in higher brain centers. CCK induces satiation when injected at meal onset, whereas MA triggers a meal in animals that are not eating, but usually fails to increase meal size. Also, MA usually triggered a meal within 10–15 min after administration, i.e., within the same time it increased celiac vagal afferent activity. In any case, further experiments are necessary to clarify this point.

Another important question is whether the proposed enterocyte energy flow monitoring mechanism differentiates between macronutrients or is tuned into a common measure of intracellular energy flow? AMPK and mTOR, the two kinases implicated in central nervous system fuel sensing [4–6], contribute in enterocytes to the control of absorption [50] and cell proliferation [51], respectively. Whether changes in enterocyte mTOR or AMPK activity affect eating is unknown, but IP injection of metformin, a potent activator of AMPK that usually reduces food intake presumably through its systemic metabolic effects, has been shown to acutely and transiently stimulate eating [52]. This is consistent with a possible role of intestinal AMPK in the control of eating.

Finally, it is unknown whether the proposed energy flow monitoring mechanism differentiates between absorbed and circulating fuels? There are marked changes in enterocyte substrate utilization in relation to eating [31, 32, 34] which suggest that luminal fuels are preferred if they are available. The capability to differentiate between luminal and blood fuels would be useful for an enterocyte energy flow monitoring mechanism to detect the meal-related nutrient fluctuation and to translate it into vagal afferent signals controlling eating behavior.

**Conclusion**

GI peptide-encoded nutrient sensing is one form of GI vagal sensing that contributes to the effect of luminal nutrients on eating [53] and metabolism [54]. An additional energy flow-sensing mechanism could provide a more direct and presumably more accurate measure of the energy available from ingested food than the indirect measure derived from GI peptide encoding [55]. To control eating efficiently, the brain needs information about the nutrient composition and energy potential of the incoming food, and it seems advantageous to obtain this information as accurately and rapidly as possible. Intestinal cells are in the ideal position to gather such information.
While enteroendocrine cells may detect the nutrient composition of a meal and prepare the body for its handling, for example by contributing to the control of insulin secretion, enterocytes appear to be well suited to monitor potential metabolic energy. They are exposed to greater fluctuations in the availability of fuels than cells in any other organ, including the brain, and the fuels that pass through the enterocytes are a good predictor of the incoming energy load. Furthermore, enterocytes see absorbed fuels earlier than any other organ. Fats in particular reach other organs only after the delay of lymphatic absorption, i.e., enterocyte recognition of dietary fat-derived energy would save significant time in providing a full energetic inventory of ingested nutrients. Last but not least, it makes physiological sense to have an energy flow monitoring mechanism in the enterocyte because nutrient absorption demands energy. In sum, the enterocyte is an attractive candidate site for peripheral energy flow sensing in the control of eating. So far mostly indirect evidence supports this hypothesis, but it deserves to be critically examined.

References


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