Dietary fat sensing via fatty acid oxidation in enterocytes: possible role in the control of eating

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Langhans W, Leitner C, Arnold M. Dietary fat sensing via fatty acid oxidation in enterocytes: possible role in the control of eating. Am J Physiol Regul Integr Comp Physiol 300: R554–R565, 2011. First published December 9, 2010; doi:10.1152/ajpregu.00610.2010.—Various mechanisms detect the presence of dietary triacylglycerols (TAG) in the digestive tract and link TAG ingestion to the regulation of energy homeostasis. We here propose a novel sensing mechanism with the potential to encode dietary TAG-derived energy by translating enterocyte fatty acid oxidation (FAO) into vagal afferent signals controlling eating. Peripheral FAO has long been implicated in the control of eating (141). The prevailing view was that mercaptoacetate (MA) and other FAO inhibitors stimulate eating by modulating vagal afferent signaling from the liver. This concept has been challenged because hepatic parenchymal vagal afferent innervation is scarce and because experimentally induced changes in hepatic FAO often fail to affect eating. Nevertheless, intraperitoneally administered MA acts in the abdomen to stimulate eating because this effect was blocked by subdiaphragmatic vagal deafferentation (21), a surgical technique that eliminates all vagal afferents from the upper gut. These and other data support a role of the small intestine rather than the liver as a FAO sensor that can influence eating. After intrajejunal infusions, MA also stimulated eating in rats through vagal afferent signaling, and after infusion into the superior mesenteric artery, MA increased the activity of celiac vagal afferent fibers originating in the proximal small intestine. Also, pharmacological interference with TAG synthesis targeting the small intestine induced a metabolic profile indicative of increased FAO and inhibited eating in rats on a high-fat diet but not on chow. Finally, cell culture studies indicate that enterocytes oxidize fatty acids, which can be modified pharmacologically. Thus enterocytes may sense dietary TAG-derived fatty acids via FAO and influence eating through changes in intestinal vagal afferent activity. Further studies are necessary to identify the link between enterocyte FAO and vagal afferents and to examine the specificity and potential physiological relevance of such a mechanism.

food intake; metabolism; energy homeostasis; small intestine; nutrient sensing

FOOD PROVIDES METABOLIC FUELS and essential nutrients, which makes eating a necessary part of the regulatory feedback loops that maintain energy and nutrient homeostasis. To fulfill this homeostatic function, eating must also be controlled by metabolic feedback. Early concepts of this metabolic control of eating focused on glucose because of its prominent role as a brain fuel and in intermediary metabolism (107, 138). Later, a common metabolic measure of energy, or energy flow, rather than a particular nutrient, was proposed to control eating (17, 48, 81, 119), and various mechanisms and locations of the crucial energy flow sensors were proposed (see Refs. 45, 86, 118). Fats account for > 30% of the energy intake of people in industrialized countries. Any energy flow sensing mechanism that influences eating should therefore also be sensitive to the oxidation of fatty acids, the major energy carriers of dietary and stored lipids. Therefore, the first demonstration of an increase in food intake after intraperitoneal injection of the fatty acid oxidation (FAO) inhibitor mercaptoacetate (MA) in rats (141) and the finding that methyl palmitoxirate (MP), another FAO inhibitor, and the glucose antagonist 2-deoxy-D-glucose stimulated eating synergistically (49) provided strong support for an energy flow sensing mechanism in the control of eating. MA primarily inhibits a group of enzymes called acyl-CoA dehydrogenases (9, 10), which catalyze the first step of mitochondrial FAO, whereas MP inhibits the carnitine palmitoyltransferase-1 (CPT-1; Ref. 49), i.e., the entry of long-chain fatty acyl-CoA complexes into mitochondria. Thus interference with two different steps of the FAO pathway similarly stimulates eating, indicating that this effect is due to the inhibition of FAO and not to obscure side effects of the administered compounds. Moreover, MP stimulated eating in rats maintained on a diet high in triglycerides comprised of long-chain fatty acids, which require CPT-1 for mitochondrial uptake and oxidation, but failed to affect food intake in rats fed a comparable diet high in medium-chain fatty acids, which do
not require CPT-1 for mitochondrial uptake (47). Again, this indicates that an inhibition of FAO can increase food intake. Subsequent studies showed that FAO inhibitors administered via different routes and under varying conditions in various species including humans (69) generally stimulate eating (see Refs. 91, 140), also supporting the notion that interference with peripheral FAO influences eating. In line with this interpretation, a stimulation of peripheral FAO with the peroxisome-proliferator receptor activator-α (PPARα) agonist fenofibrate has been shown to be associated with a reduction of food intake in obesity-prone rats (67, 88).

FAO inhibitors often stimulate eating more potently when subjects consume a fat-enriched diet (50, 69, 141), suggesting that dietary fat is sensed via FAO or that some metabolic adaption to dietary fat is necessary for the full expression of the eating-stimulatory effect of FAO inhibition. When rats treated with MA were offered a choice of macronutrients, they increased their intake of protein and carbohydrates and decreased their intake of fat, i.e., they overate the nutrients they could utilize and avoided the one they could not (150). In ad libitum-fed rats, MA triggered a meal and increased meal frequency without affecting meal size (85), suggesting that FAO contributes to the maintenance of satiety between meals rather than meal termination. After fat-rich meals, the contribution of ingested fats to the nutrient mix that is oxidized increases over time (153), and during fasting fatty acids released from adipose tissue gradually replace absorbed nutrients as fuels. Also, the eating-inhibitory effect of intravenous lipid infusions (e.g., Refs. 25, 165), which is presumably due to oxidation of the infused fatty acids (14), occurs later than the effect of isocaloric carbohydrate infusions (25). All these findings are consistent with the idea that FAO delays the occurrence of hunger. Does this mean that a decrease in FAO contributes to the physiological mechanism of meal initiation? One conceptual problem with this idea is that normally meals begin long before the endogenous fatty acid pool from adipose tissue is exhausted and before whole body FAO declines. Given the replacement of other nutrients by fats as fuels during the postprandial period, meals may actually be initiated during a gradual increase rather than a decrease in whole body FAO, indicating that the latter does not provide a physiological hunger signal. On the other hand, meal-related changes in the oxidation of dietary triacylglycerol (TAG)-derived fatty acids may occur at critical sensor sites in particular tissues, and ample evidence indicates that differences in FAO or, more specifically, differences in the organism’s capacity to increase dietary FAO-derived fatty acids in response to increases in dietary fat intake are one of the factors determining the susceptibility to dietary fat-induced overeating and weight gain (13, 65, 76, 149). A critical question, thus, is where in the body oxidation of dietary TAG-derived fatty acids is monitored to influence energy intake and expenditure.

Central vs. Peripheral Fatty Acid Sensing via Oxidation

Central nervous system FAO was first implicated in the control of eating in the mid-1980s (70, 71). Meanwhile, it is widely accepted that fat metabolism in hypothalamic neurons can influence eating (e.g., Refs. 95, 123). Energy flow in hypothalamic arcuate nucleus neurons presumably controls eating through reciprocal changes in the activity of the ubiquitous cellular energy sensor AMPK and its counterpart, the mammalian target of rapamycin (mTOR; Refs. 3, 31, 109), two fuel-sensitive kinases that appear to integrate metabolic and some endocrine signals. The discovery of these brain fuel sensors together with the putative signaling function of neuronal lipid metabolism raises the question of whether this central mechanism is sufficient or whether some peripheral, in particular diet-derived, fuel sensing is still needed for the adequate homeostatic control of eating. Currently, there is no definitive answer to this question. Yet, because the functional principles of physiological regulatory mechanisms are often similar, it may be useful to look at other homeostatic feedback loops for answers. Clearly, many of them, such as osmoregulation (18), thermoregulation (55), regulation of blood pressure (57), and blood glucose (105), include both peripheral and central monitoring of the regulated parameter and integration of all information by brain centers that control the autonomic and behavioral output. This peripheral-central hierarchy provides backup, increases efficiency of the regulation, and, hence, minimizes fluctuations of the regulated parameter. It is therefore reasonable to assume that the control of eating also relies to some extent on peripheral monitoring of diet-derived fuel utilization.

The liver, and in particular hepatic monitoring of glucose, were first implicated in the control of eating in the early 1960s (138). The available evidence from behavioral, anatomical, and electrophysiological studies now indicates that vagal afferents terminating in the wall of the hepatic portal vein (HPV) function as sensors for absorbed glucose generating signals that can influence eating (155, 157), insulin release (7, 120), and, hence, glucose metabolism (24). With respect to fatty acids, the traditional concept was that hepatocyte FAO influences eating through changes in the intracellular ATP-to-ADP ratio, hepatocyte membrane potential, and hepatic vagal afferent activity (44, 46, 61, 78, 86, 140, 156). This idea appeared to be consistent with the fact that hepatocytes mainly oxidize fatty acids to cover their own, substantial energy needs (146). It is still unclear, however, how changes in hepatocellular ATP/ADP or membrane potential should affect vagal afferent nerve activity (78, 91). A hunger or satiety signal based on electrochemical coupling of hepatocytes and afferent nerves would presumably require a consistent relation between changes in hepatocyte membrane potential, vagal afferent activity, and food intake. Such a consistent relation does not exist (78). For instance, MA and the fructose polymer 2,5-anhydro-mannitol, which decreases hepatic ATP (66), increased both food intake and hepatic vagal branch multiunit activity (97, 98), but MA decreased (96), whereas 2,5-anhydro-mannitol increased (19), hepatocyte membrane potential. Another problem is that there are barely any vagal afferent fibers in liver parenchyma (11). Although it is unclear how many vagal afferent fibers are required to relay a signal because hepatocytes are electrochemically coupled through numerous gap junctions (63), other problems remain: 1) 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, this branch carries mainly afferent fibers from the duodenum (11, 60). Therefore, neither behavioral phenomena observed after section of this vagal branch (see Ref. 78) nor electrophysiological data obtained in multunit recordings from it (19, 97, 98) can be exclusively linked to the liver. 2) Moreover, during

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fasting (20) or after administration of a β3-adrenoceptor agonist (22), MA potently inhibited hepatic FAO but failed to stimulate eating (see Ref. 79), indicating that an inhibition of hepatic FAO is not sufficient to stimulate eating. 3) Also, MA stimulated eating similarly after HPV and vena cava infusion (103). If the primary site of MA action were in the liver, a stronger effect might be predicted after HPV infusion. 4) Finally, hepatic FAO depends more on fatty acid release from adipose tissue than on dietary fat intake, unless TAG with medium-chain fatty acid are ingested. Therefore, only minimal meal-related fluctuations of hepatic FAO can be expected. In fact, labeling studies demonstrated that ingested fat reaches the liver in substantial amounts not before 2–3 h after the onset of ingestion, i.e., long after meal termination (13). With ad libitum access to food, fatty acids derived from ingested TAG may in fact become available to hepatocytes as an energy source at just about the time when a new meal begins, at least in the rat. Thus the observed associations between changes in hepatic energy status and changes in eating in response to FAO inhibition (66) need not reflect causality. In other words, the effect of FAO inhibition on hepatic energy status may just be pharmacological and not mimic a physiological change in hepatic energy status, registered by an hepatic energy flow sensor that controls eating.

Evidence for Fatty Acid Sensing by the Small Intestine

Whereas the eating-stimulatory effect of MA does not appear to originate from the liver (see above), it does originate in the abdomen. Studies using complete subdiaphragmatic vagotomy (133), systemic capsaicin administration (132, 148), celiac branch in addition to hepatic branch vagotomy (129), or subdiaphragmatic vagal deafferentation (SDA; 21), a surgical procedure that disconnects all vagal afferents from the upper gut but leaves ~50% of the efferents intact (122), all found that these manipulations blocked or at least markedly attenuated the eating-stimulatory effect of MA, indicating that this effect originates in the abdomen. Together these findings suggest the intestine as a possible site of action. Consistent with this idea, MA increased mitochondrial fatty acid content in the small intestine but not in the liver (104).

The idea that MA and perhaps other FAO inhibitors act in the intestine instead of in the liver to stimulate eating touches on the general role of gastrointestinal fat handling in energy homeostasis. Dietary fat is detected all along the digestive system, and the various mechanisms involved can substantially influence energy homeostasis: 1) fatty acids can act as taste stimuli (73, 106); CD36 and G-protein coupled receptors that are activated by fatty acids (e.g., GPR-40 and GPR120) are present on taste cells and are implicated in fatty acid tasting and fat preference (27, 89). 2) CD36 and GPR (e.g., GPR 40, 119, 120) have also been found on enteroendocrine cells, where they act as fat sensors translating fatty acid-encoded information into the release of gut hormones with their broad range of physiological effects (29, 36, 39, 58, 68, 77, 89). 3) Dietary fat also stimulates enterocytes to produce and release endogenous substances such as oleoylthanolamide (OEA; Ref. 135) and N-palmitoyl-phosphatidylethanolamine (56), which are involved in the control of fat intake. 4) The enzyme ghrelin O-acetyl transferase functions as a special fatty acid sensor in the stomach, using specific, primarily medium-chain dietary fatty acids as acylation substrates to form the biologically active form of ghrelin and thus providing another link between dietary fat and energy intake (75). 5) Finally, the intracellular intestinal fatty acid binding protein (I-FABP) has been suggested as an enterocyte lipid sensor that influences energy homeostasis (161).

In the small intestine, dietary fat is hydrolyzed to monoacylglycerol (MAG) and free fatty acids that are emulsified with phospholipids and bile acids. The resulting micelles as well as monomers of free fatty acids and MAG are absorbed by enterocytes via diffusion across the brush border membrane (reviewed by Refs. 136, 147). Monomers of free fatty acids and MAG also cross the brush border membrane by active transport, facilitated by transporters, such as CD36, FA-transport-protein-4 (FATP4), and FABP (100, 147) (Fig. 1). Interestingly, intestinal epithelial cells appear to have little acute control over the uptake of the lipid digestion products because >95% of ingested fat is absorbed (159). Once inside the cell, however, the fatty acids must be bound by FABP and taken care of rapidly, otherwise the cell risks destruction. Three types of FABP with different distributions are expressed in mammalian small intestine (1). Two of them, the liver fatty acid binding protein (L-FABP) and the intestinal FABP (I-FABP), have been linked to lipid metabolism and energy homeostasis (115, 161). Quantitatively the most important metabolic pathway for absorbed lipids is reesterification of the fatty acids and MAG to triglycerides (TAG) in the endoplasmic reticulum of the enterocyte (Fig. 1). This occurs very rapidly. In the rat, close to 80% of intraintestinally infused oleate is converted in enterocytes to biologically inert TAG within 30 s (101). The subsequent movement of TAG from the endoplasmic reticulum to the Golgi apparatus seems to be the rate-limiting step in chylomicron formation because the release of TAG into lymph is rapid as well (101). The chylomicrons are secreted from the basolateral side of the cells, where they enter the lymphatic system to eventually end up in the blood stream. The formation of diacylglycerol (DAG) from MAG and fatty acyl-CoA substrates is catalyzed by monoacylglycerol transferase (MGAT; Fig. 1). In particular, MGAT2 is predominantly expressed in small intestinal epithelial cells (172), and the MGAT pathway accounts for 75% of TAG synthesis in the small intestine; the remainder is presumably mediated by the glycerol-3-phosphate pathway. The final and rate-limiting step of TAG synthesis in both pathways is the formation of TAG from DAG by diacylglycerol acyltransferase (DGAT). Of the two isoforms of DGAT, DGAT1 and DGAT2, DGAT1 is particularly highly expressed in enterocytes (40). Often neglected or unknown is the fact that TAG are also stored in enterocytes in large cytoplasmic lipid droplets (41, 134, 175) (Fig. 1). Intestinal lipid storage is particularly important for lower animals such as Caenorhabditis elegans (6) or Burmese pythons (93) but can also be substantial in mammals. Because of their high turnover rate, enterocytes can certainly not serve as a long-term energy store, similar to adipocytes, but the temporal storage in enterocytes allows excess lipids after a fatty meal to be conserved within the cell and may also help to prevent lipotoxicity (173).

TAG from the cytoplasmic lipid droplets can be hydrolyzed and reenter the endoplasmic reticulum for TAG synthesis and chylomicron formation (173). For instance, the lipids stored in enterocytes after a fatty meal are released as chylomicrons when glucose is ingested several hours later (134), suggesting...
a close link between energy intake and enterocyte energy storage. Alternatively, fatty acids derived from enterocyte lipolysis can be oxidized in the enterocyte or used for the synthesis of other lipids (173).

Transgenic or pharmacologic interference with enterocyte lipid handling can have substantial effects on energy homeostasis. Mice with a global deletion of MGAT2 or DGAT1 have a normal phenotype on chow but decreased body weight when fed a high-fat diet, and this is mainly due to an increase in energy expenditure (151, 171). The obesity resistance of MGAT2-deficient mice (171) is presumably due to changes in intestinal fat handling because MGAT2 is almost exclusively expressed in the small intestine (172). This does not apply to findings in mice with global deletions of DGAT1, which is expressed in many tissues (23), or L-FABP, which is also highly expressed in the liver (1). However, the intestine-specific effects of DGAT1 were recently also shown in global DGAT1−/− mice engineered to express DGAT1 specifically in the intestines (90). In these mice, dietary fat secretion out of enterocytes was markedly increased and they were resistant to diet-induced obesity (90). Also of interest are findings in relation to I-FABP, which is exclusively expressed in the small intestine, where it is found along the entire length of the organ, with a maximum in the middle section (1). Male mice lacking the I-FABP are hyperinsulinemic and have a higher body weight than their wild-type controls on chow as well as on a high-fat diet, whereas female I-FABP-deficient mice are only hyperinsulinemic and do not gain weight on a high-fat diet (161). This sex difference may be related to higher intestinal and hepatic levels of L-FABP in females (99). I-FABP overexpression has recently been linked to a substantial increase in mitochondrial FAO and a concomitant decrease in lipogenesis and cholesterol transport in normal human enterocytes, suggesting a specific role for I-FABP in enterocyte FAO (111).

Together, these findings argue for a role of enterocyte FAO in energy homeostasis. Pharmacologic inhibition of chylomicron release antagonizes the otherwise strong, eating-inhibitory effect of intraintestinal fat administration under some conditions (139), and this effect may be due to blockade of apolipoprotein AIV formation (139). In contrast, pharmacologic interference with enterocyte TAG synthesis or chylomicron assembly generally inhibits eating (15, 170), and some evidence suggests that the eating-inhibitory effect of TAG synthesis blockade may be related to a metabolic shift from TAG synthesis to FAO. In our hands, DGAT1 inhibition failed to reduce food intake when rats were fed regular chow but clearly inhibited eating when rats were fed a high-fat diet, and this effect was accompanied by a metabolic shift from TAG synthesis to FAO (143). Also, DGAT1 is expressed in enteroendocrine L cells and DGAT1 knockout mice displayed an enhanced GLP-1 and
Evidence for Fatty Acid Oxidation by Enterocytes

The intestine accounts for ~25% of total body oxygen consumption (162). This mainly reflects the fact that enterocytes require substantial amounts of energy for nutrient absorption. They utilize fuels that enter the cells from the luminal and from the blood side. Glutamine and glutamate are the most important fuels for enterocytes (117, 169). In fact, glutamine utilization accounts for ~77 and 35% of CO₂ production in the fastest and fed states, respectively. This is possible because in enterocytes the activity of the enzyme glutaminase is high. It is mainly the phosphate-dependent glutaminase that converts glutamine in enterocytes to glutamate (108). Some of this glutamate is released (167, 168), but the major end product of intestinal glutamine metabolism is alanine, which is released into the HPV and can fuel hepatic gluconeogenesis (116, 117). Glutamine also provides nitrogen for the synthesis of nucleotides and other N compounds, and it stimulates the MAPK pathway and promotes enterocyte proliferation (128). Enterocytes can also utilize fair amounts of glucose. Interestingly, hexokinase activity is high in the fed state and decreases during fasting (117). Even when luminal glucose is abundant, however, enterocytes do not completely oxidize glucose (37, 169). Instead, enterocyte glycolysis generates C-3 compounds (pyruvate and lactate) for gluconeogenesis in the liver.

As already mentioned above, enterocytes can also oxidize fatty acids (37, 42, 152) that enter the cell from the serosal or the luminal side. Forty-five and sixteen percent of the fatty acids derived from the plasma or the intestinal lumen are oxidized, respectively (53). Yet, as much more fatty acids enter the cell from the intestinal lumen than from the serosal side, fatty acids derived from dietary fat provide more energy to enterocytes than fatty acids derived from the blood. Fatty acids are not an important source of energy for enterocytes when animals are fed carbohydrate-rich chow. FAO (34, 76) and even ketogenesis (34, 154) in enterocytes are markedly induced, however, when animals are fed a high-fat diet. For instance, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, the key enzyme controlling ketogenesis, is present in suckling rat intestine and disappears after weaning onto regular chow, but expression of the gene can be reinduced by only 1 wk of high-fat diet feeding (154). Interestingly, enterocyte FAO is not only induced with high-fat feeding, but the capacity of enterocytes to oxidize fatty acids also appears to be crucial for the organism’s ability to adapt to increases in dietary fat and for the propensity to become obese on high-fat diets (76). Polyunsaturated fatty acids (112, 160) and DAG (113) appear to be particularly effective inducers of enterocyte FAO, and this feature may contribute to the antiobesity effects of these compounds. Whether natural fluctuations or experimentally induced short-term changes in enterocyte FAO affect eating has not been systematically studied yet, but the fact that enterocyte FAO and the eating-stimulatory effect of FAO inhibitors both depend to some extent on dietary fat is at least consistent with such a mechanism.

Finally, significant FAO and its modulation by metabolic modifiers can also be shown in common cell culture models of enterocytes. The Caco-2 cell line, derived from a human colonic adenocarcinoma (43), shows phenotypic features of fetal colonocytes and adult, differentiated small intestinal enterocytes (137). Caco-2 cells are considered to be a good model for investigating intestinal lipid metabolism (92). Preliminary studies of ours indicate that Caco-2 cells readily oxidize long-chain fatty acids and that this process can be inhibited by the CPT-1 inhibitor etomoxir (Koss M, Langhans W, unpublished observations). Thus in vitro experiments performed with the Seahorse XF24 extracellular flux analyzer for the online measurement of aerobic respiration and glycolysis indicate that, when incubated with long-chain fatty acids, Caco-2 cells increase their oxygen consumption (indicative of an increase in FAO) by 25–40% and that this can be completely blocked by addition of the CPT-1 inhibitor etomoxir. In fact, etomoxir reduced oxygen consumption below baseline values measured before the addition of exogenous fatty acids, suggesting that there is even some baseline level of oxidation of fatty acids derived from intracellular lipid stores. On the other hand, DGAT1 inhibition, which inhibited eating in rats fed a high-fat diet (143), stimulated FAO in Caco-2 cells as well as in HuTu86 cells (16), a human duodenal adenocarcinoma cell line (142). These findings are consistent with a possible effect of enterocyte FAO on eating.

Do FAO Modifiers Influence Eating by Acting in the Small Intestine?

The CPT-1 inhibitors MP and etomoxir have been shown to stimulate eating after oral or intragastric administration in rats and humans (59, 61, 62, 69). While this would allow for an intestinal site of action, this possibility was never specifically examined, presumably because these substances were supposed to stimulate eating by inhibiting hepatic FAO. As mentioned above, we observed some indirect evidence for an effect of MA on intestinal FAO when we injected MA intraperitoneally in rats, i.e., the stimulation of eating in response to MA was accompanied by an increase in intestinal rather than hepatic mitochondrial fatty acid concentrations (104). To more directly examine whether MA acts in the intestine to stimulate eating, we investigated the effects of intrajejunal MA infusions on food intake in rats. In these experiments, different doses of MA or equivalent volumes of saline (control) were infused through previously implanted catheters into the proximal jejunum of freely moving, adult male rats fed a fat-enriched diet (40 energy-%fat; Ref. 141). The infusions were given at 3 h into the light phase of the 12:12-h dark-light cycle. Under these conditions intrajejunal MA stimulated eating and appeared to do so at lower doses than after infusion into the HPV (200 instead of 400 μmol/kg body wt; Ref. 103). This is consistent with an intestinal site of MA action to stimulate eating. Moreover, the intrajejunal MA infusions failed to stimulate eating in rats by SDA (see Table 1), indicating that abdominal vagal afferent signaling is necessary for the observed effect. These findings strongly argue for an intestinal site of action of MA. Further experiments are necessary to examine whether this also holds for CPT-1 inhibitors such as etomoxir and MP.

C75 has often been shown to inhibit eating after central and peripheral administration (e.g., refs. 30, 95), and this effect was
Table 1. Intrajejunal MA infusions acutely stimulate eating in sham but not in SDA rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham (n = 12)</th>
<th>SDA (n = 13)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.7 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>100 µmol/kg body wt MA</td>
<td>1.2 ± 0.5</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>200 µmol/kg body wt MA</td>
<td>1.6 ± 0.4*</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.8 ± 0.4</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>400 µmol/kg body wt MA</td>
<td>2.5 ± 0.4*</td>
<td>0.3 ± 0.2</td>
</tr>
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Values are means ± SE. Adult male rats were kept on a fat-enriched diet (141) and underwent subdiaphragmatic vagal deafferentation (SDA) or sham surgery as described previously (5). After recovery from surgery, infusions of mercaptoacetate (MA; 2.66 ml/kg body wt) or equivalent volumes of equiosmotic saline were given over 30 s at 3 h into the light phase in separate within-subject crossover designs for each dose and surgical group. *P < 0.05, difference between treatments is greater in sham-operated than in SDA rats, sequentially rejective Bonferroni t-test after significant ANOVA.

originally supposed to be due to the FAS-inhibiting action of C75. Some findings suggest, however, that the eating-inhibitory effect of C75 is at least in part due to the compound’s stimulating effect on CPT-1 (2). When we infused C75 (5 mg/kg body wt) as a bolus 1 h before food access into the stomach of rats adapted to an 8-h feeding:16-h deprivation schedule, we observed a substantial inhibition of eating (Jaggi T, Langhans W, Lettner C, in collaboration with Aja S, Ronnett G, Kuhajda F; Baltimore, MD, unpublished observations). C75 precipitates at low pH, and we know that fluids infused into the stomach under these conditions are rapidly emptied into the duodenum. It is therefore very possible that the administered C75 inhibited eating by acting in the small intestine. Clegg et al. (30) reported that injection of 15 mg/kg C75 intraperitoneally induced a conditioned taste aversion in rats. In our hands, C75 doses of 3.2 and 7.5 mg/kg body wt did not induce a taste aversion after intraperitoneal injection (102). We therefore think it is unlikely that a 5 mg/kg body wt dose of C75 induces an aversion after intragastric administration. The inhibition of eating after intraperitoneal C75 injection was independent of intact vagal afferents, suggesting that in this case the effect was due to a direct action of C75 in the brain (102). Several explanations appear possible for these seemingly discrepant results; it is feasible that SDA fails to block the eating-inhibitory effect of C75 after intraperitoneal administration but does so after jejunal administration. Given the complexity of the C75 molecule, it may reach enterocytes better after intrajejunal than after intraperitoneal administration. Future studies should examine this possibility or use more specific CPT-1 stimulators of eating in rats after SDA. Also, it is possible that signals generated by a stimulation or an inhibition of enterocyte FAO are relayed to the brain through different neural pathways. While the eating-stimulatory signal derived from an inhibition of FAO may travel the vagal afferent route to the brain, the eating-inhibitory signal derived from a stimulation of FAO may use nonvagal spinal, i.e., splanchnic, afferents. This possibility is suggested by findings of Sclafani et al., who demonstrated that the eating-inhibitory effects of intraduodenal maltodextrin and corn oil infusions were markedly attenuated by celiac superior mesenteric ganglionectomy (145), indicating that nutrient-derived intestinal signals do not exclusively use vagal afferents to gain access to the central nervous system neurocircuitry controlling eating or that sympathetic efferents somehow control the generation of these signals in the small intestine. A non-vagal, intestinal afferent mediation through splanchnic afferents might also explain the failure of SDA to antagonize the reduction in eating produced by DGAT1 inhibition in high-fat diet-fed animals (143). Of course, it remains to be examined whether DGAT1 inhibition reduces food intake by stimulating enterocyte FAO and whether this effect is blocked by celiac superior mesenteric ganglionectomy.

Do Intestinal Vagal Afferents Mediate the Effect of Mercaptoacetate?

Anterograde tracing studies using nodose ganglia dye injections identified three characteristic patterns of vagal terminal structures in the wall of the small intestine (12): one population with terminals in the longitudinal and circular muscle layers has been termed Intramuscular arrays, and another group of vagal afferent terminals in the myenteric plexus of the whole gastrointestinal tract is called intraganglionic laminar endings (12). Intramuscular arrays and intraganglionic laminar endings are supposed to function mainly as mechanosensors. A third population of vagal afferents without specific structures ends freely in the lamina propria of the mucosa (Fig. 1). In the rat duodenum and jejunum, these vagal afferent endings form extensive networks within the lamina propria of the mucosa of both crypts and villi (12). Their terminal axons are in close contact with the basal lamina and are thus in an ideal position to act as chemosensors, detecting various substances including absorbed nutrients and mediators released from enteroendocrine and epithelial cells.

These afferent fibers travel in the various celiac branches of the vagus. Furthermore, the so-called common hepatic branch of the vagus carries fibers innervating the proximal small intestine (gastroduodenal branch) in addition to the liver (hepatic proper branch; Refs. 11, 60). This is important in the light of behavioral phenomena observed after section of the common hepatic branch of the vagus (78, 83, 84) and electrophysiological data obtained in multiunit recordings from it (97, 98; see above). Clearly, if a major part of this common hepatic branch of the abdominal vagus innervates the proximal small intestine rather than the liver, none of these behavioral and electrophysiological findings can be exclusively linked to the liver. For instance, both the increase in multiunit vagal afferent activity by MA (97) and the attenuation of the eating-stimulatory effect of MA after common hepatic branch section (84), which were interpreted as evidence for an hepatic action of MA, may have resulted from an effect of MA on duodenal vagal afferents. Likewise, the strong eating-stimulatory effect of intraperitoneal ouabain (83), which inhibits the sodium-potassium pump, might be mediated by upper small intestinal rather than hepatic vagal afferent fibers. Randich et al. (126) observed that HPV infusion of 800 µmol/kg MA increased the activity of the common hepatic and celiac branch of the vagus similarly. Note, however, that these authors performed multiunit recordings and 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 (84, 85, 141). We (4) recently observed that 200 µmol/kg MA reliably increased the activity of serotonin-sensitive celiac vagal afferent single units when infused into the superior mesenteric artery, which sup-
plies a major part of the small intestine. Tests of the effects of intraintestinally infused MA on celiac vagal afferent activity yielded similar results, i.e., MA acutely increased single unit celiac vagal afferent activity (Arnold M, Langhans W, unpublished results). So far, however, recordings from only two fibers could be obtained with this setting because once a specific fiber is identified for the recording it is very difficult to target an intestinal load to the terminal field of this particular fiber. These results implicate the increase in celiac vagal afferent signaling in MA’s eating-stimulatory effect and are consistent with the hypothesis that an enterocyte FAO-sensing mechanism influences eating through vagal afferents, but they do not disclose whether MA increases vagal afferent activity by inhibiting intestinal FAO. A direct effect of MA on vagal afferent fibers cannot be excluded (see (97)).

Consistent with a vagal afferent mediation of MA’s effect on food intake, selective lesions of several brain areas that are involved in the central processing of vagal afferent signals have been shown to abolish the eating-stimulatory effect of peripherally administered MA. Thus lesions of the nucleus tractus solitarii/area postrema area (133), the lateral parabrachial nucleus (26), or the central area of the amygdala (131) all blocked the stimulation of eating by intraperitoneal MA, suggesting that these areas are critical components of the central nervous system pathway for eating in response to a peripheral inhibition of FAO. In line with the lesion data, MA or MP activated neurons in largely the same brain areas that proved to be critical for their effects on eating in the lesion experiments. Intraperitoneal MA or oral MP doses that stimulated eating increased the number of neurons expressing c-Fos protein, the product of the early gene c-fos, in the nucleus tractus solitarii, parabrachial nucleus, and central area of the amygdala (59, 130). An increased expression of c-Fos is a commonly used surrogate of neuronal activation. Similar to their effects on eating, the neuronal activation in response to MA and MP was also blocked by subdiaphragmatic vagotomy (130) or perivagal capsaicin administration (62).

**Possible transduction mechanisms from enterocytes to vagal afferents**

The hypothesis that enterocyte FAO produces a vagal afferent signal that influences eating raises the question how changes in enterocyte FAO can affect vagal afferent activity? Intestinal vagal afferents terminate in the lamina propria of the mucosa, i.e., these terminals are close to, but still separated from, enterocytes by a basal membrane (12). This suggests that a chemical mediator links changes in enterocyte FAO to vagal afferent activity. Several substances could serve this function (Fig. 1). 1) OEA, the amide of ethanolamine and oleic acid, is an endogenous lipid that is synthesized by enterocytes in response to fat intake and inhibits eating (94, 135). There are several overlaps between the proposed mechanisms through which OEA and MA affect eating in opposite directions: a) As MA (21), OEA affects eating supposedly through vagal afferent signaling (94). b) OEA inhibits eating by activating PPARα (94), which controls acyl-CoA dehydrogenases (28), i.e., the enzymes that are blocked by MA (9). c) MA (85) as well as OEA and other PPARα agonists (51) affect eating by changing meal frequency rather than meal size. MA may therefore stimulate eating by reducing OEA production and, hence, affecting the same downstream signaling mechanisms in the opposite direction, or OEA and MA may have otherwise overlapping intracellular signaling pathways. Whether there is such a relation between the mechanisms of both compounds’ effects on eating awaits clarification. 2) The volatile neurotransmitter nitric oxide (NO) is produced in large amounts by enterocytes. Consistent with the hypothesis that NO may act as an eating-stimulatory signal (64), inhibition of NO production is implicated in the eating-inhibitory effect of OEA (94). In turn, an increase in NO production may be involved in MA’s eating-stimulatory effect because this effect has been shown to be antagonized by the NO synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (32). It is unknown, however, whether this was a peripheral effect of NG-nitro-L-arginine methyl ester on enterocyte NO production or whether it reflected a role for NO in the central nervous system processing of the vagal afferent signal triggered by MA. Another potential link between FAO and NO is that pharmacologic activation of AMPK, which increases FAO, markedly inhibits the inducible form of NO synthase and, hence, decreases NO production (125). AMPK (164) and inducible form of NO synthase (72) are present in enterocytes. 3) The excitatory neurotransmitter glutamate, which is derived from glutamine. Enterocytes have long been known to release glutamate produced from glutamine in vivo and in vitro (167, 168). The ionotropic glutamate receptor N-methyl-D-aspartate is present on vagal and nonvagal intestinal afferents (74). Glutamate increases multiunit vagal afferent activity (121), and intraperitoneal administration of MK-801 initially blocked the eating-stimulatory effect of MA (38), suggesting that N-methyl-D-aspartate receptor activation is involved in the initial stimulation of eating by MA. 4) Ketone bodies are produced by enterocytes through FAO in response to chronic lipid exposure (34, 76, 154). D-3-hydroxybutyrate inhibits eating when administered peripherally (87) or centrally (33). The effect after peripheral administration was attenuated after hepatic branch vagotomy (82), a result that would be consistent with a local paracrine action of ketone bodies produced by enterocytes on upper small intestinal vagal afferents. MA and other FAO inhibitors have been shown to reduce circulating ketone bodies (see Ref. 140), which might be due to inhibition of intestinal in addition to hepatic FAO. These various compounds are only not mutually exclusive examples for possible chemical mediators that could link enterocyte FAO to intestinal vagal afferent signaling. Whether the release or interstitial concentration of one of these substances is in fact modulated by changes in enterocyte FAO is unknown and remains to be examined.

**Open Questions**

The hypothesis that MA, and perhaps other FAO inhibitors, stimulate eating by increasing intestinal vagal afferent activity fits the MA (130)- or MP (59)-induced increases in neuronal activation observed in several brain areas known to be involved in dietary the central processing of vagal afferent signaling (see above). However, the findings appear to be at odds with the usually strong increase in vagal afferent activity induced by gastrointestinal satiation factors such as cholecystokinin (CCK) or gastric distension (144). Eighty-eight percent of the celiac vagal afferent single units that we recorded from increased their firing rate in response to MA and CCK (4),
indicating that the opposite behavioral effects of CCK and MA are probably not mediated by stimulation of separate fibers. Yet, superior mesenteric artery MA and CCK infusions induced a markedly different pattern of neuronal activation in celiac vagal afferent fibers: whereas CCK triggered a fast (within 10–20 s), very pronounced (firing rate ~300% baseline), and transient (back to baseline after ~120 s) increase in neuronal activity, MA caused a delayed (5–10 min after infusion onset), less pronounced (firing rate ~200% baseline), and long-term (still increased at 30 min after infusion onset) response. Such different patterns of neuronal activity might well encode opposite behavioral reactions. Another not mutually exclusive possibility is that the behavioral reaction (start or stop eating) to the vagal afferent signal depends on its integration with other, context-specific inputs in the nucleus tractus solitarii or in higher brain centers. Indirect support for this interpretation is derived from the fact that the effectiveness of MA and CCK to also affect eating is situationally variable. Whereas CCK potently and reliably reduces meal size when administered at meal onset, it fails to do so when it is injected where it increased single unit celiac vagal afferent activity (4). Therefore, additional intrameal (for CCK) or between meal (for MA) signals may determine the behavioral effects of the increases in vagal afferent activity induced by the two compounds. Of course, we do not know whether the increase in celiac vagal afferent activity is instrumental in CCK’s satiating effect or whether MA also affects signaling in vagal afferents originating in the pyloric region that seem to be crucial for CCK’s satiation effect. In any case, further experiments are necessary to clarify these issues.

Another important question is whether the proposed enterocyte-monitoring mechanism in the control of eating is specific for FAO or tuned into a common measure of intracellular energy flow? As enterocytes increase FAO only when significant amounts of fat are ingested and rely otherwise mainly on glutamine/glutamate and on some glucose and ketone bodies to cover their own energy needs, one might speculate that enterocytes should be able to monitor energy flow rather than FAO oxidation (80). On the other hand, gauging the energy yield of incoming lipids already in the intestine may be particularly important for maintaining energy balance because, unlike carbohydrates and proteins, much of dietary fat bypasses the liver and reaches other potential sensor sites in the body only after the delay of lymphatic absorption. AMPK and mTOR, the two kinases involved in hypothalamic fuel sensing (31, 109), are involved in the control of epithelial transport (163, 164), cell proliferation (114), and migration in enterocytes (127). Whether changes in enterocyte AMPK or mTOR activity affect eating is unknown, but intraperitoneal injection of metformin, a potent activator of peripheral AMPK that usually reduces food intake presumably through its systemic metabolic effects, has been shown to acutely and transiently stimulate eating in rats (35). Oral administration of arginine and leucine activates the mTOR-signaling pathway in enterocytes (8, 128), but whether this translates into an inhibition of eating is unknown. Therefore, a critical examination of the role(s) of intestinal AMPK and mTOR in the control of energy homeostasis, perhaps by site-specific modifications of these two kinases, remains to be performed.

Finally, to effectively control eating, the proposed enterocyte FAO-monitoring mechanism should be able to differentiate between absorbed and circulating fatty acids. Some evidence (52) indicates that enterocytes oxidize relatively more of circulating than of luminal fatty acids. In response to a high-fat meal, however, lipid uptake from the lumen increases whereas the level of circulating fatty acids decreases. Therefore, even if the relative oxidation of luminal and serosal fatty acids does not change with a meal, the total amount of dietary fatty acids that is oxidized does increase during eating and this could have signaling function. Likewise, a decrease in the oxidation of dietary fatty acids related to the cessation of lipid absorption a few hours after a meal may provide a signal of meal initiation. Last, but not least, it is reasonable to assume that meal-induced changes in enterocyte oxidation of dietary fatty acids are particularly pronounced and particularly relevant for the proposed enterocyte-based mechanism of eating control, when enterocyte FAO is substantially upregulated in subjects adapted to high levels of dietary fat.

Perspectives

Several different mechanisms have been shown to be involved in dietary fat sensing along the digestive system, and the resulting neuroendocrine signals mediate effects of fats on eating and metabolism (e.g., Refs. 27, 29, 39, 58, 73, 75, 77, 89, 135, 166). All these mechanisms, however, provide primarily a qualitative assessment of food composition, i.e., they detect the absence or presence of the major energy yielding nutrients and prepare the body for nutrient handling, but they do not directly encode the meal’s energy content or expected yield. An additional fat-sensing mechanism based on enterocyte FAO might provide a more direct and accurate measure of the energy available from ingested fat. As outlined above, enterocytes are in an ideal position to monitor fat-derived metabolic energy and to relay this information to the brain because they are exposed to greater meal-related fluctuations in the availability of lipids than cells in any other organ, including the brain. Moreover, enterocytes see ingested fats much earlier than the rest of the body because absorbed lipids reach the general circulation only after the delay of lymphatic transport. Enterocyte recognition of dietary fat-derived energy would therefore save significant time in establishing a full energetic inventory of an ingested meal. Interestingly, intestinal gluconeogenesis has recently been proposed as another enterocyte-based metabolic mechanism involved in energy homeostasis (110). Intestinal gluconeogenesis is supposed to contribute to glucose and energy homeostasis by providing a signal for HPV glucose sensors, and it may contribute to the eating-inhibitory effect of high-protein diets as well as to the obesity-curbing and anti-diabetic effects of bariatric surgery (110, 158). Enterocyte gluconeogenesis and FAO may combine to encode energy content rather than nutrient composition of meals and relay this information to the brain. In sum, the enterocyte is an attractive candidate site for peripheral FAO sensing in the control of eating and energy homeostasis. So far mostly indirect evidence supports this hypothesis, but it deserves to be critically examined.


3. Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR. The work was supported by Swiss National Science Foundation Grant No. 31-130665.


Enterocyte fatty acid oxidation and eating


