Activation of the gut-brain axis by dietary glutamate and physiologic significance in energy homeostasis

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ABSTRACT
L-Glutamate is a multifunctional amino acid involved in taste perception, intermediary metabolism, and excitatory neurotransmission. In addition, recent studies have uncovered new roles for L-glutamate in gut-brain axis activation and energy homeostasis. L-Glutamate receptors and their cellular transduction molecules have recently been identified in gut epithelial cells. Stimulation of such L-glutamate receptors by luminal L-glutamate activates vagal afferent nerve fibers and then parts of the brain that are targeted directly or indirectly by these vagal inputs. Notably, 3 areas of the brain—the medial preoptic area, the hypothalamic dorsomedial nucleus, and the habenular nucleus—are activated by intragastric L-glutamate but not by glucose or sodium chloride. Furthermore, the chronic, ad libitum ingestion of a palatable solution of monosodium L-glutamate by rats has also been found to reduce weight gain, fat deposition, and plasma leptin concentrations compared with rats that ingest water alone. No difference in food intake was observed. Such effects may also be vagally mediated. Together, such findings contribute to the growing knowledge base that indicates that L-glutamate signaling via taste and gut L-glutamate receptors may influence multiple physiologic functions, such as thermoregulation and energy homeostasis. Am J Clin Nutr 2009;90(suppl):1S–6S.

INTRODUCTION
L-Glutamate is a multifunctional amino acid. Free L-glutamate in foods elicits a unique taste termed umami (1–3), which has been suggested to function as a sensory cue for protein ingestion (4). Inside the body, L-glutamate functions at an interface of amino acid and carbohydrate metabolism through its role as a key substrate in transamination reactions and has especially notable effects in intestinal cells (5–7), liver cells (8), muscle cells (9), placental cells (10), pancreatic β cells (11), and immune cells (12). L-glutamate also acts as the dominant excitatory neurotransmitter in the brain (13, 14) and appears also to function as a transmitter outside the brain [eg, in the enteric neurons of the gut (15)].

In addition to these roles, recent studies have identified other functions for L-glutamate. L-Glutamate receptors and their cellular transduction molecules have now been identified in gut epithelial cells (16, 17). Stimulation of these receptors by luminal L-glutamate activates vagal afferent nerve fibers (18, 19) and subsequently parts of the brain targeted directly or indirectly by these vagal inputs (eg, the cerebral cortex, basal ganglia, limbic system, and hypothalamus) (20, 21). In addition, the chronic, elective ingestion of a palatable solution of monosodium L-glutamate (MSG) by rats has been found to reduce the development of obesity, fat deposition, and elevated plasma leptin concentrations through an enhancement of energy expenditure (22). Such effects of L-glutamate may be initiated by gut L-glutamate receptors (16, 17) that are linked to afferent branches of the vagus nerve (18, 19), taste L-glutamate receptors in the oral cavity (23–27), or both. This review focuses primarily on these recent findings concerning the post-ingestive mechanisms of dietary L-glutamate.

VISCERAL MECHANISMS

Several investigators have reported that the gut epithelial cells express receptors for umami, sweet, and bitter substances that are indistinguishable from those found in the mouth (16, 17, 28, 29). The transduction elements for these receptors are also found in the same cells (17, 30–32). What roles might such “taste” receptors play in the digestive and absorptive portions of the gastrointestinal tract? Sweet receptors (T1R2 + T1R3), for example, are located on the gut enteroendocrine cells (28, 31, 32). Their stimulation by sugars and artificial sweeteners activates intracellular signaling elements such as z-gustducin and causes the release of incretin hormones such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (31). In addition to the effects on potentiation of glucose-induced insulin release, these hormones act on enterocytes to stimulate the absorption of glucose from the intestinal lumen through upregulation of Na+/glucose cotransporter 1 (32, 33) (Figure 1). In addition, glucagon-like peptide 1 exerts other significant actions, which include stimulation of insulin biosynthesis, inhibition of glucagon secretion, inhibition of gastric emptying and acid secretion, reduction of food intake, and trophic effects on the pancreas (34). Gut sweet receptors thus appear to participate in the coordination of dietary carbohydrate absorption and distribution throughout the body.

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Because L-glutamate receptors are also found in the gut epithelial cells (16, 17), might these receptors also participate in physiologic systems that control nutrient digestion, absorption, metabolism, and distribution in the body? Here we describe the existence of an L-glutamate-sensing system in the gut linked to activation of vagal afferent fibers that transmits food-related signals to the brain.

**L-GLUTAMATE RECEPTORS IN THE GUT**

Currently, 2 candidate L-glutamate receptors have been identified in the cells lining the gut: metabotropic L-glutamate receptor type 1 is located in the chief cells (pepsinogen-secreting cells) of the stomach (16), and a heterodimer L-glutamate taste receptor, T1R1 + T1R3 (taste receptor 1, subtypes 1 and 3), is found in epithelial cells of the stomach, small intestine, and colon (17). The cellular transduction molecules of taste L-glutamate receptors, including α-gustducin, phospholipase Cβ2, and transient receptor potential channel M5, are also found in these cells (17), which indicates the presence in these cells of the complete receptor transduction machinery. These findings suggest that tastelike L-glutamate receptors in the gut might detect ingested L-glutamate on the luminal side of the gut and provide this information to adjacent cells and neurons.

**ACTIVATION OF ABDOMINAL VAGAL AFFERENT FIBERS**

The abdominal vagus nerve consists of 3 branches—gastric, celiac, and hepatic—each containing both afferent and efferent fibers. The administration of MSG into the stomach, duodenum, and portal vein has been found to activate vagal afferents of the gastric, celiac, and hepatic branches, respectively (18). Such findings suggest the existence of L-glutamate-sensing mechanisms at these sites (stomach, duodenum, and liver). Of the 3 vagal components, gastric afferents respond specifically to L-glutamate (MSG): ie, no response is elicited by infusing any of the other amino acids (or NaCl) into the stomach (19) (Figure 2). In contrast, hepatic afferents respond to all amino acids delivered into the portal vein (35). The response of gastric afferents to MSG is concentration dependent (19). Moreover, the...
vagus does not respond to the intravenous administration of MSG (19). Therefore, the effect of the luminal l-glutamate administration is local (because any leakage into the circulation would be without effect). The afferent endings of the vagus are not in direct contact with gut epithelial cells. How, then, does the activation of l-glutamate receptors stimulate vagal nerve endings? We have observed that the activation of gastric vagal afferents by intragastric MSG can be suppressed by either 1) luminal perfusion with lidocaine, a local anesthetic; 2) pretreatment of animals with p-chlorophenylalanine to deplete endogenous serotonin (5-HT) stores in the gut; 3) administration of granisetron, a selective antagonist at 5-HT receptor type 3; or 4) administration of Nω-nitro-L-arginine methyl ester [a nonselective inhibitor of nitric oxide synthase (NOS)] (19). Moreover, the vagal response to MSG can be mimicked by intragastric administration of sodium nitroprusside (an nitric oxide donor), an effect that is abolished by pretreatment with the 5-HT receptor type 3 antagonist granisetron (19). Together, these findings suggest that luminal l-glutamate activates the vagal nerve indirectly via the production and release of nitric oxide and the release of 5-HT.

Anatomical and immunohistochemical evidence reveals that 5-HT-immunoreactive cells are found in the stomach, particularly in the superficial part of the mucosal epithelium and at the base of the fundic glands (36). Immunoreactivity for 5-HT receptor type 3 is localized in the neck of the fundic glands (36). NOS1/neuronal NOS-immunoreactive cells of bipolar shape are found in the lamina propria, where a dense network of neuronal cells is present. These findings suggest that complex cellular events mediate intragastric l-glutamate signaling.

Some evidence indicates that gut l-glutamate signaling via the afferent vagus is functionally important. In particular, behavioral data in vagotomized rats suggest that vagal function influences elective MSG ingestion. Selective abdominal vagotomy reduces the ingestion of MSG solutions (240–600 mmol/L) in the order of total vagotomy > gastric vagotomy > celiac vagotomy > hepatic vagotomy = intact controls (20, 37). Because the effects of gastric vagotomy are comparable with those of subdiaphragmatic total vagotomy, the effect on MSG ingestion appears to be mediated principally through the gastric branches. In contrast to MSG ingestion, vagotomy does not affect the ingestive behavior of proline (a sweet amino acid) at any concentration (Figure 3), which suggests that the vagotony-induced reduction of MSG intake is not related to a local unpleasant sensation in the gut.

**BRAIN RESPONSES TO LUMINAL GLUTAMATE**

It has repeatedly been suggested that the sensing of protein ingestion by animals might be linked to the l-glutamate content in foods (4). It is unlikely that such protein “sensing” would be tied (at least exclusively) to l-glutamate taste in the mouth because taste is limited to free, but not to protein-bound, L-glutamate, and the free l-glutamate content of natural food items is not tied to their protein content (eg, tomatoes are high in free l-glutamate, but not protein) (3, 38). But the total l-glutamate content of foods (free + protein bound) might provide a reasonable index of protein ingestion because l-glutamate is the most abundant amino acid in almost all dietary proteins (38). Because the ingestion of foods high in protein (and thus l-glutamate) does not lead to appreciable changes in plasma l-glutamate concentrations, the body (the brain) is unlikely to monitor protein intake via meal- or diet-related variations in plasma l-glutamate. However, luminal l-glutamate contents, once proteins are digested, may well reflect protein ingestion (eg, reference 39) and thus might constitute a reliable signal for protein intake. In this case, the stimulation of gut l-glutamate receptors by luminal l-glutamate after protein digestion and the subsequent activation of afferent vagal fibers might be a mechanism by which the brain senses protein ingestion.

We have therefore studied brain responses to luminal l-glutamate concentrations. The afferent vagus projects onto neurons in the caudal part of the nucleus of the solitary tract from which projections arise that spread to numerous areas of the brain (41). Some of these brain areas might be responsive to activation of the afferent vagus by luminal l-glutamate. We measured the brain responses to the intragastric administration of taste substances [glucose (sweet), MSG (umami), and sodium chloride (salty), each at 60 mmol/L] by using functional magnetic resonance imaging in rats. Sixty millimoles per liter was selected because it is the concentration of MSG most preferred by rats (20, 37). The rats were anesthetized and placed into the magnetic resonance imaging animal holder in a fixed position, and solutions were infused by a stomach tube beginning 30 min after the scanning commenced (which produced blood oxygenation concentration-dependent images). Stomach infusions lasted 10 min, and images were collected for 60 min after infusions began (21). Nutrient infusion activated a number of brain areas, including the cortex, basal ganglia, limbic system, and hypothalamus (20, 21). All of these regions receive vagal information through the nucleus of the solitary tract (41). Notably, the medial preoptic area, dorsomedial nucleus of the hypothalamus, and habenular nucleus were activated only by MSG, whereas the nucleus accumbens was activated only by glucose. The amygdala was activated by both glucose and MSG. Other areas, such as the insular cortex (primary visceral sensory cortex), anterior cingulate cortex, caudate-putamen, hippocampus, and lateral
hypothalamic area were activated by MSG, glucose, and sodium chloride. Both the medial preoptic area and the dorsomedial nucleus of the hypothalamus have been proposed to play a role in thermoregulation (42–44), whereas the habenular nucleus, especially the lateral habenula, participates in emotive decision making by influencing the activity of midbrain dopamine and 5-HT neurons (45, 46). The amygdala is a key structure in the evaluation of the biological importance of foods (47, 48). Such findings suggest that luminal L-glutamate may play a role in thermoregulation, energy homeostasis, reward processing, and emotional behavior.

The temporal patterns of the brain responses (functional magnetic resonance imaging images) were also distinct for each luminal stimulus. The glucose response developed slowly, with the peak at 20–30 min after the beginning of gastric infusion; the response was sustained up to 60 min (21). This response pattern paralleled the changes in blood glucose concentrations. However, the MSG-induced response developed rapidly, with the peak occurring just at the end of the 10-min infusion period. The response then dropped off to baseline values within the next 15 min. The functional magnetic resonance imaging response to sodium chloride infusion paralleled (in time) that for MSG but was much weaker. In another experiment, 150 mmol NaCl/L produced almost no response (21), which suggests that the response to 60 mmol NaCl/L was likely a hypotonic effect (49) because the experimental conditions (gastric expansion, temperature change, and anesthetic) were otherwise the same for each solution tested. An analysis of the response to MSG (60 mmol/L) minus that to sodium chloride (60 mmol/L) shows a result for MSG similar to that of MSG alone (although reduced). Together, these results suggest that gut luminal L-glutamate may be “sensed” in the brain via a signal provided by the afferent vagus.

In addition to these findings, we have recently observed that the brain activation induced by L-glutamate, but not by glucose, is abolished after subdiaphragmatic total vagotomy (50). This finding suggests that afferent vagus signaling is critical to gut L-glutamate “sensing” in the brain, but not to gut glucose sensing by the brain. The metabolic fate of glucose and L-glutamate after ingestion is shown in Figure 1. Presumably because of its importance as an energy source in many cells in the body, and especially in the brain, gut epithelial cells metabolize relatively little glucose after the absorption of glucose (6, 7). In contrast, most luminal L-glutamate (and glutamine) is metabolized in the small intestine (5–7). As a consequence, blood glucose concentrations increase after the ingestion of glucose, whereas blood L-glutamate concentrations generally do not rise after the ingestion of glutamate (unless a very large dose is administered) (5, 20, 51). Before absorption, luminal glucose stimulates “sweet” receptors in the gut, which triggers the release of incretins into the blood (31), whereas L-glutamate stimulates L-glutamate receptors to activate the vagus nerve (through the release of nitrous oxide and 5-HT) (19). In concert with the incretins, the elevation of blood glucose by glucose ingestion enhances the release of insulin from pancreatic β cells (31, 34). Hence, the dominant pathway by which glucose ingestion signals the brain appears to be the circulation (ie, a humoral pathway involving the direct sensing of blood chemicals by the brain), whereas the pathway by which L-glutamate signals the brain is primarily neural, ie, vagus mediated. If this difference holds, it is interesting to note that the mechanism by which the gut manages ingested glucose (with relatively little metabolism) and L-glutamate (with almost complete metabolism) harmonizes with the manner in which each signals the brain regarding its presence in the gut.

![FIGURE 4](image-url)

**FIGURE 4.** Spontaneous ingestion of monosodium L-glutamate (MSG) solution suppresses obesity in rats. (Left) A rat drinking water alone (water group); (middle) A rat drinking 1% MSG solution and water (MSG group); (top, left and middle) abdominal fat images scanned by using magnetic resonance imaging; (bottom, left and middle) rats in an anesthetized condition; (right) MSG intake does not modify food intake and lean mass but reduces plasma leptin concentrations and fat mass (both subcutaneous and intraabdominal fat masses). **Significantly different from control, *P* < 0.05 and **P* < 0.01. Reproduced with permission from references 20 (left and middle) and 22 (right), respectively.
EFFECTS OF ORAL γ-GLUTAMATE ON BODY ENERGY METABOLISM

We recently studied the effect of oral MSG ingestion on energy balance in growing rats (22). Because MSG generally enhances the palatability of food, we were interested in assessing whether MSG ingestion increased food intake and promoted the development of obesity. In this study, rats were given free access to 2 water bottles, one containing a 1% (w/vol) MSG solution [the most preferred concentration of MSG for most rat strains (20, 37)] and the other water (22). Rats given free access to MSG and water showed a high preference (93–97%) for the MSG solution. Interestingly, rats ingesting the MSG solution showed less weight gain over the 15-wk study, compared with rats ingesting no MSG. In addition, abdominal fat mass and plasma leptin concentrations were lower in rats ingesting MSG than in animals ingesting water only (Figure 4). Naso-anal length, lean mass, food and energy intakes, blood pressure, blood glucose, and plasma concentrations of insulin, triglyceride, total cholesterol, albumin, and γ-glutamate did not differ between the 2 groups. Taken together, these results suggest that MSG ingestion reduces weight gain, body fat mass, and plasma leptin concentrations in growing rats. These changes are most likely mediated by increased energy expenditure because food intake was not affected by MSG ingestion. Supporting this possibility are published data indicating that the ingestion of an MSG solution enhances the thermic effect of foods (52, 53). Conceivably, such effects of MSG might include neural links that involve the afferent branches of the vagus nerve in the gut, the afferent sensory nerves in the oral cavity, and perhaps activation of the medial preoptic area and dorsomedial nucleus of the hypothalamus, areas that are activated by gastric MSG (20, 21) and are known to be involved in thermoregulation (42–44). Such possibilities need to be examined.

CONCLUSIONS

γ-L-Glutamate is an important biomolecule. Its roles in taste (umami) perception, intermediary metabolism, and excitatory neurotransmission have been well documented. In addition, evidence from recent studies suggests new functions for dietary γ-glutamate. In particular, dietary γ-glutamate is now known to stimulate γ-glutamate receptors in the stomach and intestines, which produces local actions on gut function, and also, through the release of signaling molecules (nitric oxide and 5-HT), activates the afferent vagus nerve and consequently a number of areas in the brain. One current hypothesis is that this signaling cascade informs the brain of the amount of protein ingested (20). In the other study, the chronic intake of a palatable solution of MSG has been found to slow the development of obesity, fat deposition, and plasma leptin concentrations in growing rats, most likely through an enhancement of energy expenditure (22). Altogether, these findings indicate that dietary γ-glutamate influences numerous physiologic functions, which suggests a broad, integrative role for dietary γ-glutamate in body homeostasis. (Other articles in this supplement to the Journal include references 54–82.)

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REFERENCES


