Dietary Glutamate Signal Evokes Gastric Juice Excretion in Dogs

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Abstract
Background: Dietary-free L-glutamate (Glu) in the stomach interacts with specific Glu receptors (T1R1/T1R3 and mGluR1–8) expressed on surface epithelial and gastric gland cells. Furthermore, luminal Glu activates the vagal afferents in the stomach through the paracrine cascade including nitric oxide and serotonin (5-HT). Aim: To elucidate the role of dietary Glu in neuroendocrine control of the gastrointestinal phase of gastric secretion. Methods: In Pavlov or Heidenhain gastric pouch dogs, secretion was measured in the pouch while monosodium glutamate (MSG) was intubated into the main stomach alone or in combination with liquid diets. Results: In both experimental models, supplementation of the amino acid-rich diet with MSG (100 mmol/l) enhanced secretions of acid, pepsinogen and fluid, and elevated plasma gastrin-17. However, MSG did not affect secretion stimulated by the carbohydrate-rich diet and had no effect on basal secretion when applied in aqueous solution. Effects of MSG were abolished by denervation of the stomach and proximal small intestine with intragastrically applied lidocaine and partially suppressed with the 5-HT3 receptor blocker granisetron. Conclusions: Supplementation of amino acid-rich liquid diets with MSG enhances gastrointestinal phase secretion through neuroendocrine pathways which are partially mediated by 5-HT. Possible mechanisms are discussed.

Introduction

In recent years, the application of molecular cell approaches has shed new light on the mechanisms of the gastric phase of gastric secretion. According to the classical concept, gastric phase secretion is induced by mechanical distention and chemical stimulation of the stomach wall with nutrients which involve the interplay of neural, endocrine, and paracrine pathways that match secretion to quantity and quality of food in the lumen [1]. Although it was known for a long time that amino acids and short peptides are the most potent components of food stimulating gastric phase secretion of acid and gastrin release [2, 3], specific sites of interaction of amino acids with gastric gland cells were unknown. In the last
decade, several sensors of amino acids have been described on the apical and basolateral membranes of gastric mucosal cells, including calcium-sensing receptors (CaSRs) [4, 5], metabotropic glutamate receptors (mGluRs) [6, 7], the T1r1/T1r3 receptor dimer previously identified as an umami taste receptor [8], and amino acid transporters [9]. CaSRs expressed in gastric acid-secreting parietal cells and pepsinogen-secreting chief cells [10] as well as surface mucus-secreting cells [11] and gastrin-secreting G-cells in the antrum [12] are sensitized by aromatic, polar and acidic amino acids, with aromatic being the most potent [4]. Immunohistochemical analysis of rat stomach sections revealed that mGluR1 is found in the lower region of gastric glands at the apical membrane of chief cells and possibly in parietal cells [6]. Recent studies of various cell-enriched fractions from the isolated rat gastric mucosa revealed vaster distribution of mGluRs. Parietal and chief cell fractions expressed T1R1 and mGluR1 and a fraction of smaller endocrine cells containing D- and A-cells expressed mGluR2–4,7 [7].

Glutamate receptors in the stomach mucosa likely contribute to nervous and paracrine control of stomach functions. Among the other 20 natural amino acids only L-glutamate evokes firing in the afferents of gastric branches of the vagus [13]. Activation of vagal afferents is preceded by secretion from mucosal cells of nitric oxide followed by serotonin (5-HT) which in turn interacts with 5-HT3 receptors on afferent fibers [13, 14].

Previous studies of the physiological role of dietary glutamate in the gastrointestinal tract (GIT) did not specify the site of its action, presuming a primary role for taste perception. Free L-glutamate or monosodium salt of glutamate (MSG) in food elicits fifth basic taste sensation known as ‘umami’ [15], which is considered as a cue signal of protein ingestion. Numerous umami taste receptors on the tongue convey excitation via gustatory nerves to the brainstem nuclei where they mediate reflexes of the cephalic phase of GIT responses. Orally applied glutamate stimulates the secretion of saliva, bile and pancreatic juice [16-18] as well as secretion of insulin [19]. Supplementation of liquid diets with MSG promotes gastric emptying in humans [20] and in dogs [21].

Several groups of investigators have already shown that free glutamate in food stimulates gastric exocrine and endocrine functions. Nevertheless, a special role of this amino acid in the cephalic phase of gastric secretion seems arguable. MSG or the combination of MSG and inosine 5’-monophosphate (IMP) added to a meaty meal caused a rapid and prolonged increase in gastric juice secretion compared with a control diet in dogs [22]. Similar effects of dietary-free glutamate were observed in clinical studies, when supplementation of hospital meals with MSG improved gastric acid secretion and appetite in patients with chronic atrophic gastritis. Furthermore, ingestion of MSG elevated blood gastrin level both in patients and intact dogs [23]. However, sham feeding of food flavored with MSG did not affect gastric acid secretion and blood gastrin level in humans [24], and stimulation with MSG of the back of the throat of dogs produced lower gastric secretion than other basic tastes [25].

The present study was designed to evaluate the role of dietary-free glutamate in the regulation of the gastric phase of gastric secretion. In particular, we focused on nerve signaling which could be stimulated in the stomach by luminal glutamate. To distinguish neuroendocrine responses to MSG from local activation of exocrine cells, we used models of small gastric pouches according to Pavlov (vagally innervated) or Heidenhain (vagally decentralized) prepared in dogs. An ability of dogs as of many other mammalian species to discriminate umami taste of MSG or 5’-ribonucleotides was confirmed earlier [26]. In the applied models, test solutions were intubated into the main stomach avoiding the oral cavity, whereas gastric secretions of acid, pepsinogen and fluid were measured in the isolated gastric pouch of the mucosa of which had no contact with the solutions. We monitored secretion in the gastric pouch for 2 h after application of test solution. It is quite clear that during this period, solutions from the main stomach were totally evacuated to the intestine. The half-time of gastric emptying of liquid meals in dogs ranged between 4.5 and 43 min depending on viscosity [27] and did not depend on body surface area, mass, age or sex animals [28]. Therefore, we consider that it is correct to term the observed response as gastrointestinal phase of gastric secretion rather than the gastric phase.

Materials and Methods

In the experiments, we used 11 adult mongrel dogs of both sexes weighing 22–25 kg. Surgery and experimental procedures were approved by the Animal Care and Use Committee of the Pavlov Institute of Physiology. Dogs were anesthetized with ketamine and xylazine (75 and 25 mg/kg, i.m., respectively) and prepared with small vagally innervated gastric pouches according to Pavlov (n = 7) or vagally decentralized pouches according to Heidenhain (n = 4). Pouches approximately of one volume of about 50 ml were made from tissues of the greater curvature of the corpus and connected with the abdominal wall by the fistula. A modified Thomas cannula was placed into the fistula and its proximal part made of Plexiglas was sutured to the pouch wall.
An outer part of the cannula made of titanium was exteriorized through the abdominal wall and left unplugged throughout the study to ensure drainage of the pouch. A smaller cannula for infusion of test solutions was implanted into the main stomach and its outlet was capped.

Experiments were performed after an 18-hour fast, during which water was allowed, and were repeated no more often than twice weekly. Throughout the experiment, effluent from the pouch was collected by gravity during 15-min periods into glass tubes attached to the outlet of the pouch cannula. 5 min before interchange of tubes, a pouch was rinsed with 5 ml of saline (140 mmol/l NaCl, 37°C, pH 6.0) to ensure washout of secreted gastric juice. During stabilization three 15-min samples were collected; the third one was used to calculate a baseline secretion. After baseline secretion was obtained, 20 ml of test solution was infused into the main stomach and secretion in the pouch was monitored for a further 2 h. Only one intragastric application was done in each experiment.

Total acidity of an effluent was evaluated using pH/PCO2 measurements by summarizing free acidity derived from the value of pH with concentration of dissolved CO2, assuming that CO2 in gastric juice arose from a reaction between H+ and HCO3 [29]. Pepsinogen concentrations in samples were determined spectrophotometrically at 280 nm by degradation of horse hemoglobin standard at pH 2.5. Fluid production was calculated by subtracting of the weight of the rinsing saline (5 g) from the weight of the sample. Total output of acid and pepsinogen per 15 min was calculated by multiplying concentration by volume of a sample. Peripheral venous blood samples were collected at the end of the stabilization period and 30, 60, and 120 min after infusion of solutions into the main stomach. Concentration of gastrin-17 was determined in plasma using ELISA Kit (Biohit, Helsinki, Finland). Total gastrin-17 output was calculated as area under the time-concentration curve.

The following chemicals and high caloric liquid diets were used in the study. MSG, IMP and liquid diets were produced by Ajinomoto Co., Inc. (Tokyo, Japan). Before each experiment, MSG and IMP were dissolved in distilled water and the pH of the MSG solution was adjusted to 6.0 with 0.1 N HCl. According to the manufacturer, the amino acid-rich (Elental) diet contained (g/100 ml): 4.69 amino acids (consisting of: Ile, Leu, Lys-HCl, Met, Phe, Thr, Trp, Val, His-HCl, Arg-HCl, Ala, Asp, Glu, Glu, Gly, Pro, Ser, Tyr), 21.13 dextrin, 0.169 fat, minerals and vitamins. The carbohydrate-rich diet included (g/100 ml): 23.48 dextrin, 0.31 plum flavor, and aspartame. In the experiments, liquid diets were applied at equal caloric concentrations (1 kcal/ml). Granisteron (Kytril) from Hoffmann-La Roche Ltd, Switzerland, was injected intravenously (i.v.); and pentagastrin (Sigma, USA) was administered subcutaneously (s.c.). Both were diluted before injection up to 3 ml with sterile saline. The local anesthetic lidocaine (Moskhimfarm Ltd, Russia) was applied into the main stomach 30 min before stimulation of gastric secretion.

Data are presented as the mean ± SEM (n = number of experiments). Net production was calculated as total output during 2 h after treatment following subtracting of the base level secretion. Net productions were compared with paired or unpaired Student’s t test as appropriate. Values of p < 0.05 were considered to be statistically significant.

Results

In the Pavlov pouch, basal secretions of acid, pepsinogen and fluid were 24.3 ± 4.7 μmol/15 min, 190.7 ± 18.4 μg/15 min, and 0.34 ± 0.07 ml/15 min (n = 24), respectively. Basal secretions of acid, pepsinogen and fluid in the Heidenhain pouch were much lower: 7.2 ± 2.4 μmol/15 min (p < 0.01), 72.6 ± 26.6 μg/15 min (p < 0.01), and 0.16 ± 0.09 ml/15 min (p < 0.05, n = 12). Infusion into the main stomach of 20 ml of aqueous solutions of MSG (100 mmol/l) or IMP (10 mmol/l) separately or in combination did not influence basal secretion in both models. In preliminary studies, it was shown that application of 20 ml of saline did not stretch the main stomach sufficiently to stimulate secretion in the Pavlov pouch.

In the Pavlov pouch, high-caloric amino acid-rich diet (Elental, 20 ml, 20 kcal) caused an increase of acid and fluid output reaching a peak 30–45 min after infusion and lasting up to 2 h. However, the Elental diet did not change the baseline pepsinogen secretion. Supplementation of the diet with 100 mmol/l MSG markedly enhanced net production of acid (from 72 ± 23 to 254 ± 46 μmol/l/2 h; p < 0.01, n = 16) and fluid (from 2.44 ± 0.33 to 5.04 ± 0.72 ml/2 h; p < 0.01, n = 16). The effect of MSG (10–100 mmol/l) was concentration-dependent and for acid secretion reached statistical significance at 50 mmol/l. It is particularly noteworthy that enrichment of the diet with 100 mmol/l of MSG provoked a net secretion of pepsinogen (305 ± 89 μg/l/2 h). In the Heidenhain model, MSG (100 mmol/l) also caused potentiation of gastrointestinal phase secretion induced by the Elental diet. Secretion of acid was enhanced from 25 ± 9 to 166 ± 32 μmol/2 h (p < 0.01, n = 8). Additionally, MSG provoked a net pepsinogen output (369 ± 94 μg/l/2 h). Furthermore, when 100 mmol/l of MSG was added to the Elental diet, the plasma level of gastrin-17 was increased by about 30% from 22 ± 5 to 34 ± 6 pmol/l/2 h (p < 0.05, n = 8).

To check whether effects of MSG depend on sodium ions, the control diet was co-applied with NaCl in an amount equimolar to MSG. Addition of sodium did not alter the secretory response in concentration up to 100 mmol/l. IMP, which produces in taste buds a synergistic effect with L-glutamate, did not modify gastrointestinal secretion, when 10 mmol/l of IMP was applied in combination with MSG.

Temporary denervation of the main stomach and likely denervation of the proximal small intestine was performed in the Pavlov model with a local anesthetic, lidocaine (5%, 10 ml), infused into the main stomach 30 min

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before application of the test solution. Pretreatment with lidocaine totally suppressed the potentiating effect of MSG on diet-induced secretion and abolished the increase in plasma gastrin-17. Interestingly, denervation significantly enhanced net production of acid stimulated by glutamate-free Elental diet from 72 ± 23 to 250 ± 91 μmol/2h (p < 0.01, n = 16), but had no influence on secretion of fluid and pepsinogen. In addition, after pretreatment with lidocaine there was a tendency for an increase of diet-dependent production of gastrin-17.

Recently it has been shown that antagonists of 5-HT_3 receptor attenuate glutamate-specific impulse discharge in gastric branches of the vagus nerve associated with stimulation of mucosal mGluR1 [13]. In our study, a selective blocker of 5-HT_3 receptors, granisteron (20 μg/kg, i.v.), injected 30 min before the diet, had no influence on basal secretion from the Pavlov pouch. However, granisteron reduced the potentiating effect of 100 mM MSG on pepsinogen and fluid output (p < 0.05; n = 6), although it did not affect the increase in acid production.

Carbohydrate-rich diet (20 ml, 20 kcal) administrated into the main stomach stimulated the gastrointestinal phase of secretion in the Pavlov pouch similar to the effect of the Elental diet, but had no effect in the Heidenhain model. However, MSG (100 mmol/l) added to the carbohydrate-rich diet failed to enhance the secretory response. To find out whether the effect of dietary glutamate depends on the interplay with other nutrients at the luminal side of gastric mucosa, we used the Heidenhain model to assess the effects of MSG on secretion stimulated by pentagastrin (1 μg/kg, s.c.). Application of aqueous MSG solution (20 ml, 100 mmol/l) into the main stomach simultaneously with injection of pentagastrin caused an increase of acid secretion as compared to control, when 100 mmol/l of NaCl was applied (p < 0.05, n = 8). In contrast, pentagastrin-stimulated secretions of pepsinogen and fluid were not altered by MSG.

Discussion

Current understanding of the physiological role of dietary-free L-glutamate in the stomach relies largely on data generated in immunohistochemical and electrophysiological studies, which showed expression of mGluR2 on membranes of exocrine and endocrine cells of gastric glands [6, 7] and also described a special ability of luminaly applied glutamate among the other natural amino acids to induce firing in gastric branches of the vagus [13]. In the present study, we tested the hypothesis that stimulation of neural pathways by intra gastrically applied MSG could modulate gastric secretion.

In awake fasted dogs, supplementation of an amino acid-rich liquid diet with MSG enhanced gastrointestinal phase of secretion of acid, pepsinogen and fluid and increased plasma levels of gastrin-17 at doses similar to the concentrations used for food seasoning [30]. Furthermore, intragastrically applied MSG potentiated secretion induced by pentagastrin. However, application into the main stomach of aqueous MSG solution alone did not affect basal secretion in the pouch, suggesting that MSG modulates secretion induced by other nutrients, rather than triggers it. A corresponding role of MSG was recently reported in the control of gastric emptying in humans. Enrichment of a protein-rich liquid diet with MSG promoted gastric emptying, whereas an aqueous solution of MSG did not influence the emptying rate [20].

In our study, the stimulatory effect of MSG fully depended on the innervations. Increases in gastric secretion and plasma gastrin-17 levels caused by intragastrically applied MSG were abolished after temporary denervation of the main stomach and likely the proximal small intestine with the intragastrically infused local anesthetic, lidocaine. Additionally, we have shown that effect of MSG (namely to increase pepsinogen and fluid output) is partially mediated by 5-HT_3 receptors, which is consistent with the finding that 5-HT is the paracrine factor linking luminal sensing of free glutamate and activation of vagal afferents in the stomach [13]. It is important to note that denervation markedly enhanced the gastrointestinal phase of gastric secretion and the plasma gastrin-17 levels stimulated by the amino acid-rich diet itself (glutamate-free). Moving from the stomach to the duodenum, dietary amino acids could induce both stimulation and inhibition of gastric exocrine secretion. Interacting with G-cells of gastric glands, amino acids activate release of gastrin enhancing gastric secretion independently of the innervation [1]. In the small intestine, dietary amino acids induce output of cholecystokinin (CCK) from mucosal cells, which activates intestinal vagal afferents inhibiting gastric secretion [31]. Lidocaine likely suppresses intestinal afferentation and thus reduces central inhibition of gastric secretion. This suggests that activation of vagovagal circuits by intragastric glutamate produces additional stimulation of gastric secretion and probably counteracts the mechanisms of central inhibition of gastric secretion. On the other hand, we should keep in mind that intragastric free glutamate could interact with gastric gland endocrine cells independently of nervous pathways. Metabotropic glutamate receptors are...
expressed on gastric gland D-cells producing somatostatin [7], a potent inhibitor of gastric secretion. Somatostatin levels are upregulated by a number of factors coupled with digestion, e.g. increase of acidity, gastrin, CCK, etc. [32]. Glutamate-sensing receptors in D-cells are coupled to a Gi protein that should inhibit somatostatin secretion, resulting in stimulation of gastric exocrine secretion [7].

It is important that the effect of dietary MSG on the gastrointestinal phase of gastric secretion depended upon the characteristics of the co-applied macronutrients. Glutamate significantly enhanced secretory responses to the amino acid-rich diet (dextrin-based), but did not affect responses to an equicaloric amount of dextrin alone (carbohydrate-rich diet). A corresponding selectivity of the interaction of glutamate with liquid diets was demonstrated for gastric emptying. Enrichment of protein liquid diet with MSG promoted gastric emptying, but was ineffective when a carbohydrate diet was administrated [20]. We suggest that the effect of MSG could depend upon the proportion of neural and humoral signals in the stomach and small intestine during ingestion of different nutrients. In the duodenal lumen, proteins and amino acids produce greater postprandial release of CCK than nutrients. In the duodenal lumen, proteins and amino acids are major stimulators of intestinal release of the peptide tyrosine-tyrosine, inhibiting many gastrointestinal functions, including gastric acid secretion and gastric emptying [33]. Luminal application of carbohydrates is a strong stimulus for release of glucagon-like peptide-1 slowing gastric emptying and producing short-term augmentation of postprandial glucagon secretion which in turn could stimulate gastric acid secretion by vagus-dependent mechanisms [32].

In conclusion, we showed that sensing of dietary-free glutamate by the gastric mucosa could enhance the gastrointestinal phase of gastric secretion via specific neuroendocrine pathways which are different from those of other amino acids. The effect of dietary-free glutamate depended on characteristics of co-applied nutrients; it potentiated secretion induced by intragastrically administrated amino acids but had no influence when co-applied with dietary carbohydrates. The stimulatory role of glutamate fully depends on the stomach innervation and partially involves 5-HT3 receptors characteristic for afferent nerve endings of the vagus. We suggest that free glutamate fortification of liquid diets could improve gastric secretory capacity and should be taken into account during enteral nutrition.

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Disclosure Statement

No conflict of interest exists.

References

References


