Luminal amino acid sensing in the rat gastric mucosa

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Submitted 27 December 2005; accepted in final form 6 June 2006

Recently, it has been shown that taste transduction-related molecules, such as α-gustducin and a family of bitter-sensing taste receptors (T2Rs), were also expressed at the GI mucosa (14, 46), suggesting that taste receptors could be possibly involved in gut nutrient sensing. Likewise, receptors for well-known gut hormones [i.e., serotonin (5-HT), cholecystokinin (CCK) and VIP] are also expressed in taste buds of the oral cavity, and it has been proposed that these gut hormones could modulate taste perception too (13, 15, 16, 38). Taking all these observations together, it seems as if the tongue and the upper GI tract share common systems for nutrient sensing.

We were the first to find that the gastric branch of the vagus nerve in the rat responded to the intragastric administration of the monosodium salt of glutamic acid, which is a major component of many proteins in foodstuffs (26, 27); that led us to hypothesize that the stomach could “taste” ingested nutrients through chemical sensing systems similar to the one functioning in the tongue and intestine. The novelty of this conjecture resides in the fact that, to date, the gastric wall has been only considered to be able to detect meal volume by mechanical stretch, whereas nutrients, indicators of meal quality, were assumed to be mainly recognized at the intestine mucosal wall, which chemical information would need to be transported to the brain through the afferent fibers of the vagus nerve. A variety of data support this vagovagal reflex elicited by the intestinal mucosal chemoperception (2, 21, 23, 26). However, besides this work, to our knowledge no reports were found in relation to the existence of gastric mucosal chemical sensors linked to the vagal nerve electrophysiological stimulation. Carbohydrate (glucose) and lipid (fatty acids) chemical perception in the gut, where the intercellular cross talk conveys the luminal nutrient signal to the vagus nerve, was clearly demonstrated for the duodenal wall (19, 47). On the other hand, nobody has shown until now the mechanisms at work for the gastric chemical sensing of amino acids at the stomach mucosa. In this study, we showed that the electrophysiological response of afferent fibers from the gastric branch of the vagus nerve were specifically activated in response to glutamate on the rat gastric mucosa via an intrinsic cascade involving nitric oxide (NO) and 5-HT. As a result, we proposed a new hypothesis based on the gastric perception of the free glutamate that is found in foodstuffs.

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats, weighing around 250–330 g, were used for this experiment. Rats were housed under controlled temperature (23°C) and light cycle (lights on between 0700 and 1900) and were fasted for 18 h with free access to tap water before...
surgery. The surgical techniques and other experimental methods are documented extensively elsewhere (26, 41). Under urethane anesthesia (1 g/kg ip), the left carotid artery was cannulated with a heparinized polyethylene catheter (200 U/ml heparin in saline) to monitor blood pressure, and the left femoral vein was cannulated for drug administrations. After the pylorus of the stomach was closed with silk suture, a polyethylene catheter was inserted into the fundus. A balloon (15 mm × 13 mm; Physio-Tech, Tokyo, Japan) connected to the catheter was inserted into the stomach from the esophagus to measure distension-evoked vagal responses. Solutions for the intragastric perfusion experiment were applied at a flow rate of 1 ml/min with a peristaltic pump through a catheter inserted into the fundus. The output was captured by another catheter placed through the pylorus. All solutions were kept at 37°C during gastric perfusion.

Under a dissection microscope (Olympus SZX12, Tokyo, Japan), the nerve bundle of the left gastric branch was split with a sharp blade, leaving a length of ∼3 mm. Fine vagal filaments were dissected from the main nerve trunk and placed on a silver hook recording electrode. The perineural connective tissue was placed on a reference electrode. All recordings were made from the peripheral cut end of the vagal nerve still innervating the stomach. The abdominal wound was covered with a saline-moisturized gauze, and rats maintained at 37°C over a heating pad (BWT-100, BRC, Nagoya, Japan).

Electrophysiological recordings. The electrode was connected to a head stage (JB-101J, Nihon-Koden, Tokyo, Japan) and the signal was differentially amplified 10,000 times before being filtered with a bandwidth of 150 Hz to 1 kHz (SEN-6000, Nihon-Koden). The neural signal output, together with the signal from the pressure transducer, was acquired by a PowerLab interface (PowerLab, ADI instruments) and viewed online by a Macintosh computer running Chart software. The nerve signal was digitally sampled at 4 kHz, which was sufficient to allow spike discrimination. Nerve activity was analyzed either by conversion of raw data to standard pulses and counting (5-s bin width) or by an integration of the raw data with a 1-s time constant by using an off-line software such as spike histogram extension (SHE; ADI instruments) and Spike 2 (Cambridge Electronic Design). Data were also recorded on tape for later analysis. Afferent nerve activities were characterized in terms of average discharge frequency (spikes/s, 5-s bin width). Baseline discharge was determined over a 4-min period just before drug administrations, whereas the maximum response was determined as the increase in discharge above baseline during a 5-s period identified as the peak in afferent nerve activity in a sequential rate histogram.

Experimental protocol. After 15-min baseline recording for signal stabilization, the variability of the afferent activity was assessed with a bolus injection of 10 μg/kg 5-HT (iv). Afferent nerves that failed to respond to the 5-HT stimuli were discarded. All experiments were performed at least 20 min after checking for 5-HT response. Baseline discharge was determined over a period of 4 min just before the start of each experiment. For the mechanical response study, each stimulus (1–4 ml) was applied at a rate of 0.1 ml/s with 20 min interval, the maximal increases in discharge rate above baseline within 2 min (Peak) and the mean discharge rate at 10 min (Stable) just after gastric distension were measured. For nutrient response studies, each amino acid (2 ml) was injected at a flow rate of 0.1 ml/s. Glutamate response appeared to be a biphasic one. This consisted of an initial transient increase in discharge rate above the baseline (primary rise), within 2 min of the response, that was followed by a long-lasting activation (secondary rise). The mean discharge rate that increased above the baseline 20 min after the onset of the experiment was considered as a secondary rise index. For the intragastric perfusion study, each perfusate was applied at a flow rate of 1 ml/min while monitoring intragastric pressure. Before experiments were started, we estimated the intragastric effects of various pHs (saline at a pH of 5, 6, or 7 adjusted with 0.05 N HCl or NaOH) and osmolarities (10, 150, 450, and 1,000 mmol/l NaCl solutions) on the gastric afferent activities. The average discharge rates of pH 5, 6, and 7 were 9.5 ± 0.7, 9.9 ± 0.6, and 9.6 ± 0.8 spikes/s, respectively (n = 3). The rates of 10, 150, and 1,000 mmol/l NaCl were, respectively, 10.0 ± 0.5, 9.8 ± 0.6, 9.5 ± 0.6, 9.8 ± 0.9, 9.4 ± 0.4 spikes/s (n = 3).

Nutrients and chemical agents. All amino acids were made by Ajinomoto (Tokyo, Japan). Hydrochloride salts of basic amino acids (lysine and arginine) and monosodium salts of acidic amino acids (glutamic acid and aspartic acid) were used for the present experiment. The pH of each amino acid solution (150 mmol/l) was between 5.2 and 6.9. Chemical agents such as 5-HT, sodium nitroprusside (SNP), SB39604 hydrochloride, cimetidine chloride, atropine chloride, lidocaine, and p-chlorophenylalanine (PCPA) were purchased from Sigma, and N<sup>0</sup>-nitro-L-arginine methyl ester (L-NAME) was purchased from Tocris. Granisetron was from Chugai Pharmaceutical (Osaka, Japan). All chemicals except SB39604 and PCPA were dissolved in a saline solution just before experiments. SB39604 was dissolved in ethanol (10 mg/ml; stock solution), and PCPA was suspended in 0.5% carboxy-methyl-cellulose-Na.

Statistics. Data were expressed as means ± SE (n is the number of animals). The half-maximal inhibitory dose was estimated by a conventional least-squares fitting procedure to a mirror image of the Michaelis-Menten equation. ANOVA was performed by using non-parametric tests such as the Wilcoxon’s signed-rank test or the Kruskal-Wallis test, as appropriate. A probability of P < 0.05 was considered statistically significant.

RESULTS

Vagal afferent activation by gastric distension. First, we recorded the responses from the afferents of the ventral gastric vagus specifically responsible for distension. Gastric distension was achieved by filling a balloon catheter inserted into the stomach with a saline solution (1–5 ml). This stretch stimulus evoked a monophasic vagal afferent activation in a volume-dependent manner (Fig. 1A). The half-maximal effective volume for a peak response was 1.64 ml. The distension-induced increase of the vagal firing rate declined exponentially to basal firing rates even after maintaining the distension stimuli. When the injected volume was below 2 ml, the afferent nerve activation returned to baseline within 120 s following the distension. Data for volume-dependent responses of afferent fibers was summarized in the Fig. 1B.

Before the experimental assessment for amino acids, we checked the effects of a 5-HT<sub>3</sub> antagonist (granisetron) on the mechanosensitive component of the afferent activation. As shown in Fig. 1C, intravenous administration of granisetron (10 μg/kg) attenuated the basal and 5-HT-evoked afferent firings by 20 and 77%, respectively (n = 4). However, the distension-induced response (2 ml saline) was not blocked by the 5-HT<sub>3</sub> antagonist at all.

Responses of the ventral gastric afferents to luminal glutamate and its specific action among 20 amino acids. Intragastric administration of an isotonic 150 mmol/l solution (2 ml) of the L-amino acid monosodium glutamate evoked an afferent nerve response with marked different latencies and time course activation, which could be clearly distinguished from the primary response of the balloon-induced distension (Fig. 2). This mechanical stretch provoked an initial afferent nerve response that was transient, characteristic of mechanosensitive fiber stimulation. However, the afferent nerve response that glutamate-evoked increased gradually and occurred after the initial transient activation. This secondary rise seems to reflect the activation of chemosensitive fibers as previously reported elsewhere (26, 27). The response from the onset of the exper-
The first activation (primary rise) lasted for 42 ± 2.5 s, whereas from the onset to the secondary activation the interval was of 507 ± 66 s, respectively (n = 5), which continued for more than 1 h before returning to the baseline. The concentration-dependent response to glutamate at the secondary afferent reaction was summarized in the Fig. 2B. This shows that afferent discharge rates above baseline were dose dependently increased 20 min after each dose of glutamate application (50, 150, and 450 mmol/l). We also examined whether a low concentration of glutamate (10 mmol/l) was able to evoke the activation of gastric afferents. Without a doubt, a slight increase in the vagal afferent discharge was observed even at this low dose of glutamate (10 ± 0.6 and 18.3 ± 3.3 spikes/s for pre- and postglutamate application, respectively) (n = 4; P < 0.05, Wilcoxon’s signed-rank test). Therefore, the threshold concentration for glutamate had to be below this amount of 10 mmol/l. In contrast to luminal application, the intravenous administration of glutamate failed to increase the afferent discharge at a dose of 30 mg/kg (data not shown; n = 3). This observation strongly indicates that the stimulation of gastric afferents depends explicitly on the presence of glutamate in the lumen of the stomach since the elevation of plasma circulatory glutamate does not have any effect.

Next, vagal afferent responses for 19 amino acids other than glutamate were studied. For this experiment, an aqueous solution of each amino acid at a concentration of 150 mmol/l was introduced into the rat stomach, and the afferent nerve activity of the gastric branch was continuously monitored. The maximum increase of the afferent discharge rate at the chemosensitive secondary rise after each amino acid application was summarized in the Fig. 3. This study revealed that gastric vagal afferents could only be significantly activated with the luminal introduced into the rat stomach, and the afferent nerve activity of the gastric branch was continuously monitored. The maximum increase of the afferent discharge rate at the chemosensitive secondary rise after each amino acid application was summarized in the Fig. 3. This study revealed that gastric vagal afferents could only be significantly activated with the luminal

**Fig. 1.** Mechanosensitive responses of ventral gastric afferents. A: representative recording of gastric afferent discharge displayed as a sequential rate histogram in response to 2-ml distension. Vertical bar indicates 50 spikes/s. B: volume-dependent effects on the gastric afferent responses. Peak, maximum increase in the discharge rate during the gastric distension; Stable, constant increase in the discharge after decaying the peak response. Each point and vertical bar represents mean ± SE from 4 different animals. C: effect of granisetron (Gran.) on the mechanosensitive vagal afferent response. Granisetron (10 μg/kg) was intravenously applied ~4 min before applying 2-ml distension. 5-HT, serotonin. Inset: typical sequential rate histogram. Each column and vertical bar represents mean ± SE from 4 different animals. ***P < 0.05 (Wilcoxon’s signed-rank test).

**Fig. 2.** Gastric afferent responses stimulated by luminal glutamate. A: representative recording of gastric afferent discharge displayed as a sequential rate histogram after intragastric administration of 150 mmol/l (2 ml) glutamate. Actual wave recording at each point was also displayed. B: dose-dependent effects of luminal glutamate on the secondary afferent activations. Each glutamate solution (50, 150, and 450 mmol/l, 2 ml) was introduced into the rat stomach, and the mean discharge rate above baseline at 20 min was plotted. Each column and vertical bar represents mean ± SE from 4–6 rats.

**Fig. 3.** Gastric vagal afferent responses to intragastric application of various amino acids (AAs). Each aqueous solution (150 mmol/l, 2 ml) was introduced into the rat stomach, and the mean discharge rate above baseline at 20 min was plotted. Each column and horizontal bar represents mean ± SE from 5 different rats. **P < 0.05 vs. saline (Kruskal-Wallis test).
administration of glutamate; none of the other amino acids that constitute common proteins of our body induced a similar response \((P < 0.01\) vs. saline; Kruskal-Wallis test). In particular, it should be noted that l-aspartate, glycine, alanine, and serine did not induce the secondary afferent activation. All those amino acids are known to interact with brain glutamate receptors.

Receptor pharmacology involved in the luminal glutamate-triggered afferent response. The secondary phase, the glutamate-specific response, was selectively attenuated by either the pre- or posttreatment with the 5-HT\textsubscript{3} antagonist granisetron (Fig. 4, A and B). However, granisetron could not inhibit the first phase in the afferent response, which was the result of gastric distension \((28.6 \pm 6.9\) spikes/s in control rats and \(26.1 \pm 8.5\) spikes/s in 10 \(\mu g/k g\) granisetron-treated rats; \(n = 5\)). Intravenously, granisetron \(\left(10 \mu g/k g\right)\) inhibited the secondary glutamate specific response by 76.6 \pm 5.8\%, and its half-maximal inhibitory dose was 0.23 \pm 0.10 \(\mu mol/kg\) \((n = 4; \text{Fig. 4C})\). The residual component that was insensitive to granisetron was not affected by intravenous administrations of either 10 \(\mu g/k g\) ketanserin \(\left(5-HT_2\right.\) receptor antagonist), 100 \(\mu g/k g\) SB39604 \(\left(5-HT_4\right.\) antagonist), 100 \(\mu g/k g\) lorglutamide \(\left(CCK_A\right.\) receptor antagonist), or 100 \(\mu g/k g\) cimetidine \(\left(H_2\right.\) receptor antagonist) \((n = 2;\) data not shown). Thus luminal glutamate responses were predominantly mediated by 5-HT\textsubscript{3} receptors at the afferent nerve endings.

Glutamate signaling pathway in the gastric mucosa. Subsequently, to determine the source of 5-HT responsible for the activation of vagal afferents, we investigated the effect that 5-HT depletion may have to the glutamate-specific response using PCPA. It is well established that PCPA depletes 5-HT stores in the intestine \((45, 47)\). In Fig. 5, gastric afferent responses induced by the luminal glutamate between normal and PCPA-treated rats were compared \((each n = 5)\). Moreover, rats that received PCPA \((200 mg/kg ip injections twice/day for 2 consecutive days)\) were also contrasted with vehicle \((0.5\%\) carboxy-methyl-cellulose-Na\) treated rats. 5-HT depletion with PCPA did not inhibit the first transient \(\left(\text{stretch-evoked}\right)\) component at all but instead abolished the secondary sustained \(\left(\text{glutamate specific}\right)\) component of the afferent discharges. To make sure that during those experiments afferent nerve endings were intact, we applied 5-HT intravenously and estimated the responses of the vagus nerve; reaction to 5-HT was not significantly different between the PCPA-treated and vehicle-treated rats \((data not shown, n = 3)\).

It has been shown that NO synthases \((\text{NOSs})\) as well as 5-HT are densely distributed in the upper GI tract \((7, 31)\). As a result, the effect of a nonselective NOS inhibitor, \(\text{L-NAME}\), was studied to evaluate whether NO was involved in the vagal glutamate response. Figure 6A shows that \(\text{L-NAME}\) \((10 mg/kg iv)\) influenced the luminal glutamate-induced vagal afferent response and arterial blood pressure as well. The last one, blood pressure, rapidly increased by \(\sim 41\) mmHg within 10 min after the administration of \(\text{L-NAME}\) \((98 \pm 6.1\) to 139 \pm 10 mmHg; \(n = 3\); \(P < 0.05\), Wilcoxon’s signed-rank test). Under these experimental conditions, the intravenous application of acetylcholine \((1 \mu g/kg iv)\) could not reverse the effect of \(\text{L-NAME}\) by rapidly reducing blood pressure \((data not shown, n = 3)\). This reflects that \(\text{L-NAME}\) treatment efficiently blocked endothelial NOS. Using the same experimental conditions, but this time with the intragastric injection of 150 \(\mu mol/l\) glutamate \((2 ml)\), only the component of the transient nerve discharge could be stimulate, which was equivalent to the distension-induced response \((\text{Fig. 6B})\). The vagal responses to luminal glutamate in normal rats and \(\text{L-NAME}\)-treated rats were quantitatively summarized in the Fig. 6B. Results indicate that the inhibition of intrinsic NO release was not able to block the first transient \(\left(\text{distension-evoked}\right)\) component at all but could abolish instead the second sustained \(\left(\text{glutamate specific}\right)\) component of the afferent discharges of the vagus nerve.

Nevertheless, in contrast to the activation observed for glutamate, the effect of \(\text{L-NAME}\) itself or the blockade of intrinsic NO release did not affect directly the response of

![Fig. 4. Effects of granisetron on the luminal glutamate-induced afferent activation. Pretreatment effect (A) and posttreatment effect (B) of granisetron \((10 \mu g/kg iv)\) on the glutamate response. Glutamate aqueous solution \((2 ml; 150 \mu mol/l)\) was applied to the rat stomach. Typical recording of gastric afferent discharge was displayed as a sequential rate histogram. C: dose-dependent inhibition of granisetron for the luminal glutamate-induced afferent activation. Each value means relative percentage against maximal inhibition value using 1 \(\mu g/kg\) granisetron. Each point and vertical bar represents mean \(\pm\) SE from 4 different rats.](http://ajpgi.physiology.org/)

![Fig. 5. Effect of mucosal 5-HT depletion on the luminal glutamate-induced afferent responses. A: representative recording of gastric afferent discharge displayed as a sequential rate histogram in the p-chlorophenylalanine (PCPA)-treated rat. PCPA was intraperitoneally applied twice per day for 2 days at a dose of 200 mg/kg. 5-HT was intravenously administered at a dose of 10 \(\mu g/kg. B: gastric afferent responses induced by 150 \mu mol/l luminal glutamate in the vehicle-treated (○) and PCPA-treated rats (●). Each point and vertical bar represents mean ± SE from 4 different rats.](http://ajpgi.physiology.org/)
gastric vagal afferent to 5-HT. Vagal afferents reacted to an intravenous bolus of 5-HT (10 μg/kg) with 37.6 ± 7.5 spikes/s before L-NAME treatment. Fifteen minutes after the intravenous injection of L-NAME (10 mg/kg), the response of afferents was 47.8 ± 14.9 spikes/s (n = 3).

Effects of intragastric perfusion with NO donor and 5-HT on the gastric afferents. To verify that mucosal NO itself could trigger gastric vagal activation, we examined the luminal effect of a NO donor, SNP, on gastric afferents. We could prove first that the intragastric luminal perfusion of 100 μg/ml SNP evoked the afferent activation of the gastric ventral vagal nerves (Fig. 7A) and that an intravenous injection of L-NAME (10 mg/kg) was intravenously applied 10 min before intragastric administration of 150 mmol/l glutamate. B: gastric afferent responses induced by 150 mmol/l luminal glutamate in the control (○) and L-NAME-treated rats (●). Each point and vertical bar represent mean ± SE from 4 different rats.

Depressed the glutamate response in a time-dependent manner, so that after 20 min, glutamate response was down to 80% (n = 3). We showed elsewhere that the discharges of afferent fibers induced by 5-HT (10 μg/kg iv) were also depressed by the application of lidocaine over the mucosa of the stomach without affecting the stretch-gated afferent nerve component (41). Thus the luminal glutamate sensing by the gastric vagus seemed to be a local event within the gastric mucosa.

DISCUSSION

In the present experiment, we demonstrated a possible mechanism for the detection of glutamate in the mucosal wall of the stomach. This mechanism triggers the secretion of 5-HT after glutamate stimulation via a mucosal NO release, which in turn activates the 5-HT3 receptors at the afferent nerve endings that innervate the stomach. To date, most of all nutrient detection mechanisms of the upper GI tract have intensively focused in the study of the duodenal mucosa (19, 23, 24, 47). Our report is the first one, in our understanding, that acknowledges the existence of an amino acid (glutamate) sensing cascade in the gastric wall.

According to our experimental conditions, in the present study, distension- and glutamate-mediated components of the luminal glutamate-sensing by the rat gastric vagus.
afferent responses from the gastric branch of the vagus nerve were clearly discriminated. The distension-induced electrophysiological stimulation of the vagus nerve was transient with an exponential decay (Fig. 1) and also insensitive to the 5-HT\textsubscript{3} receptor antagonist granisetron, whereas the intragastric administration of glutamate stimulation evoked a long-lasting nerve discharge followed by a rapid transient component (Fig. 2). That first transient component was not inhibited by granisetron, but the receptor antagonist did strongly block the secondary long-lasting component instead (Fig. 4). Thus the macroscopic stretch- and glutamate-mediated components of the afferent responses of the vagus nerve could be separated by their time-dependent kinetics or their pharmacological sensitivity to the firing pattern.

What is more, we showed that the luminal activation by glutamate of the gastric branch afferents from the vagus nerve took place via serotonin secretion through NO production. This was based on the following evidence: 1) afferent electrophysiological responses at the gastric branch of vagus nerve after glutamate stimulation were specifically blocked by perfusion of the local anesthetic lidocaine (10 mmol/l) on the lining of the stomach. This directly suggests that sensing of glutamate detected by the vagus nerve occurred within the gastric mucosa. 2) 5-HT depletion with a tryptophan dehydroxylase inhibitor, PCPA (Fig. 5), reversed this effect of the afferent activation by luminal glutamate. Likewise, the firing rate of afferent nerve fibers under the stimulation with glutamate was also specifically blocked by a 5-HT\textsubscript{3} receptor antagonist (Fig. 4 and 5). In summary, these observations are strong indicators for considering that the gastric afferent fibers of the vagus nerve were not activated directly with the presence of glutamate on the surface of the stomach mucus but stimulated instead by 5-HT, which could have been released from primary sensing cells within the mucosa, perhaps enterochromaffin (EC) cells. 3) Finally, the effect of glutamate was also prevented by blocking the release of NO with l-NAME treatment while the intragastric perfusion of a NO donor evoked the activation of 5-HT\textsubscript{3} receptors (Fig. 6 and 7). For that reason, it is believed that glutamate stimulation of the gastric mucosa works by inducing 5-HT release with the consequent activation of its 5-HT\textsubscript{3} receptors at the gastric vagal nerve endings via stimulation of a local NO. This process refers to the glutamate induced NO and 5-HT all around the gastric mucosa.

Neurons that innervate the lining of the stomach can produce at the same time NO and 5-HT, which regulate gastric motility as well as gastric acid secretion through the modulation of intrinsic nerve activity (18, 20, 40). Nonetheless, neurons are not the only source of these bioactive molecules. There is also a large amount of nonneuronal 5-HT and NO in the gastric mucosa itself (4, 30, 31), and we feel that the physiological roles for those molecules in the stomach have not been clearly identified yet. This present report suggests that gastric nonneuronal 5-HT and NO can act in the stomach mucosa as paracrine substances as well after the recognition of nutrients, such as an amino acid like glutamate, in a process similar to the one reported for the duodenal glucose sensing (47). In addition, mucosal NO has been implicated in different components of gastric mucosal defense, including increases of the mucosal blood flow and mucin secretion, activation of mucosal immune cells, and repair of the epithelia after injuries caused during digestion (3, 7). Furthermore, it is thought that the diffusion of NO within the mucosa or into its basal microvessels might be effective in local disinfection or in the maintenance of microcirculation because of its antiplatelet effect. Thus nonneuronal 5-HT and NO in the gastric mucosa might also regulate gastric digestion, by acting as a transmitter for vagal amino acid sensing and a paracrine hormone.

Present observations strongly point out the direct or indirect serotonin-containing cell stimulation (EC cells or mast cells) after the local NO production in the gastric mucosa. This final 5-HT release activates, as a result, the afferent endings of the vagus nerve. In the colon of guinea pigs, NO release was reported to be mediated by the the 5-HT of the EC cell (17). However, to date, we could not find any report supporting the role of exogenous or endogenous NO as a gastric inducer for 5-HT release (32). Many of the reports referring to NO assign this substance a stimulatory effect toward the GI exocrine and endocrine systems. For instance, NO donors stimulate histamine secretion in rodent EC-like cells (12) and somatostatin release from rabbit D cells (1) as well as pepsinogen release from guinea pig chief cells (8). Altogether, it is plausible to consider the existence of a direct cascade starting from NO to follow with 5-HT release of EC cells in the rat gastric mucosa.

In the regard for glutamate, its physiological function in the GI tract has started to be investigated. For instance, we know now that oral intake of monosodium glutamate stimulates exocrine secretion (saliva and gastric, bile, and pancreatic juices) (28, 29, 33, 39, 43) as well as the GI endocrine system such as insulin (11). Moreover, the infusion of monosodium glutamate into the stomach directly evokes a gastric vagovagal reflex accompanied by insulin secretion (27). These reports strongly suggest that not only gastric but also taste stimulation by glutamate might be, in part, involved in those cephalic responses.

On the gut chemical perception, Fujita and collaborators (9) originally proposed the paraneuron-hypothesis that claims the existence of neuron-like cells that can sense nutrients from the foodstuffs and send signals in response. This hypothesis was based on the histochemical observation in which gut endocrine cells were bipolar cells extending an apical process to the gut lumen and releasing their messenger substances from the cell base in response to the apical stimuli. These bipolar endocrine cells could be also observed in the gastric antrum, but their possible involvement in the chemical perception of nutrients by the vagus has never been discussed. Afferent fibers from the gastric branch of the vagus nerve increase their electrophysiological activity only at the intragastric application of glutamate, not with any of the remaining amino acids tested (Fig. 3). This seems to be specific for the chemical amino acid sensing in the stomach since it has been well documented that fibers from the celiac nerve could respond to almost all of amino acids applied into the duodenum (23). What are the candidate molecules for sensing glutamate at the surface of the stomach? At the oral cavity, several glutamate-sensing receptors have been identified (T1R1-T1R3 heterodimer, ionotropic, and different variants various metabotropic glutamate receptors) (6, 25, 35), but it is still unknown whether those molecules are expressed on the rat gastric mucosa. In the present experiment, a potent agonist for the NMDA receptor, aspartic acid, failed to stimulate the vagal afferent fibers from the gastric branch (Fig. 3). This suggests that the NMDA receptor is not be involved in this vagal response. We need to look further into the identity of
the possible receptors that would function as glutamate sensors on the gastric mucosa.

As summary, we showed in this work that glutamate at the surface of the stomach could evoke a mucosal chemical cascade led by NO release and followed by 5-HT to trigger at the end a postprandial visceral sensation, which probably regulates food intake and digestion. It seems that glutamate in the GI tract could be “tasted” at the surface of the stomach. Our proposed regulatory pathways may help to specify the role of glutamate to maintain body nutrition homeostasis through the regulation of the vagus nerve electrophysiological activity at the afferent terminal fibers that innervates the GI tract. Glutamate is one of the most abundant amino acids in plant and meat protein foodstuffs, and it is found in a free form that coexists with those proteins at relatively high concentrations. After tomatoes ripen or cheese fermentation takes place, the content of glutamate can reach up to or more than 20 mmol/l, whereas traditional seasonings such as soy and oyster sauce contain more than 60 mmol/l of glutamate (10, 44). Under the assumption that 10 mmol/l of glutamate was enough to evoke a significant increase for the firing rate of afferent nerve fibers, it could be realistically considered that the electrophysiological activation of the terminal afferent nerve endings that innervate the stomach mucosa could occurred under the influence of an activation of the terminal afferent nerve endings that innervate the stomach mucosa. We believe that the complete elucidation to a better understanding of food digestion regulation and ingestive behavior control. Why it is important? Along this line of research, the knowledge in the presence of specific regulatory receptors in the stomach mucosa will open the door for a new horizon in nutrition and new ways of intervention inside the field of gastroenterology that, at the end, may bring a whole unique approach for the development of new therapeutic drugs.

ACKNOWLEDGMENTS

The author thanks for Dr. Yoshie at Department of Physiology II, Nippon Dental University, for helpful comments on the manuscript.

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