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Existence of NO-triggered vagal afferent activation in the rat gastric mucosa

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ABSTRACT

Aim: We previously reported the possible involvement of mucosal nitric oxide (NO)-triggered 5-HT release in luminal glutamate sensing by the gastric vagus nerve, and we proposed that the stomach, like the duodenum, could "taste" luminal nutrients. Nitric oxide synthase (NOS) is widely distributed in the gastric mucosa, but the physiological role of mucosal NO is not well understood. In this study, we investigated the functional coupling of NO and vagal nerve endings in the gastric mucosa.
Main methods: For electrophysiological recordings, male Sprague-Dawley rats were anesthetized with urethane, and afferent nerve responses of rat vagal gastric branches to a NO donor were monitored.
Key findings: Intravenous application of 100 μg/kg sodium nitroprusside (SNP) transiently increased afferent nerve discharges of the rat ventral gastric vagus, which was followed by rapid changes in blood pressure. High doses of SNP (>300 μg/kg, i.v.) showed a biphasic increase in afferent discharges. Secondary activation of the vagal afferent continued even after blood pressure returned to basal levels. SNP-evoked afferent responses were abolished by mucosal 5-HT depletion using *p*-cholorophenylalanine and were inhibited by pre- and post-treatment with the 5-HT_3 antagonist granisetron.
Significance: These pharmacological results strongly indicate that NO-triggered 5-HT release is coupled to vagal afferent activation in the rat gastric mucosa.
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Introduction

A series of neural and humoral mechanisms regulates gastrointestinal functions that precede, accompany, and follow food intake and digestion. It is well established that sensory perception by the abdominal vagus initiates and sustains coordinated processes of gastrointestinal motility, circulation, absorption, exocrine and endocrine secretion, immunity, and satiation (Buchan 1999; Furness et al. 1999; Schwartz 2000; Travagli et al. 2003). The epithelial cells in the gastrointestinal mucosa are primary sites for the perception of nutrients and chemicals in dietary foods. Similarly, mucosal cells and vagal afferent nerves form a similar chemical sensing system in the GI tract. Indeed, vagal afferents express a wide range of membrane receptors that can modulate their sensitivity (Grundy 2004; Kirkup et al. 2001), and luminal carbohydrates stimulate 5-HT release from enterochroamaffin cells in the duodenum, which is then sensed by vagal afferents via 5-HT_3 receptor activation (Zhu et al. 2001). In addition, luminal lipids stimulate vagal afferents by releasing mucosal CCK and 5-HT from the duodenal mucosa (Lal et al. 2001). Thus, it is clear that several primary mechanisms for epithelial perception must exist in the duodenum.

Luminal glutamate activates gastric vagal afferents as well as celiac afferent nerves (Niijima et al. 2005). We recently reported that taste-sensing receptors such as a novel variant of metabotropic glutamate receptors (mGluRs) are distributed in the rat gastric mucosa (San Gabriel et al. 2007), and intra-gastric application of glutamate activates brain nuclei related to feeding behavior and gut function in a vagal nerve-dependent manner (Tsurugizawa et al. 2009). In the rat stomach, luminal glutamate stimulates vagal afferent nerve activity by triggering NO and 5-HT release from the gastric mucosa (Uneyama et al. 2006). Mucosal 5-HT acts as a common transmitter during gastric nutrient-sensing as well as during duodenal sensing. Nitric oxide (NO) is also an essential molecule that facilitates food storage by inducing gastric distention and protects gastric mucosa during digestion by increasing mucosal blood flow and mucin secretion (Brown et al. 1993). Moreover, it promotes the repair of mucosal epithelia injured by the digestive process (Calatayud et al. 2001; Cho 2001). We previously proposed that the stomach can "taste" glutamate-containing foods by a novel nutrient-sensing system, which transfers luminal glutamate information to vagal afferents via chemical signals such as NO and 5-HT (Uneyama et al. 2008). NO is well known as a mediator of mucosal microcirculation (Moncada et al. 1991), but there is no direct evidence to suggest that NO can induce vagal afferent activation via 5-HT release from the rat gastric mucosa. In the present study, we examined the effects of a NO donor on gastric vagal afferents and investigated whether vagal afferents are activated by mucosal NO-triggered 5-HT release in the rat stomach.

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Material and methods

Animal preparation

Male Sprague-Dawley rats weighing 250–330 g were used for this experiment. The rats were housed in a controlled temperature environment (23 °C) and a regular light cycle (lights on at 07:00–19:00). They were fasted for 12 h with free access to tap water before surgery. The experiments were approved by The Committee for Animal Experiments at Ajinomoto Inc. and were carried out in accordance with the guidelines of the United States National Institutes of Health. Under urethane anesthesia (1 g/kg, i.p.), the left carotid artery was cannulated with a heparinized polyethylene catheter (200 U/ml heparin in saline) for blood pressure monitoring, and the left femoral vein was cannulated for drug administration. Under a dissection microscope (Olympus SZX12, Tokyo, Japan), the nerve bundle of the left gastric branch was sectioned lengthwise into 3 mm segments with a sharp blade. Fine vagal filaments were dissected from the main nerve trunk and placed on a silver hook recording electrode, with perineural connective tissue placed on a reference electrode. Recordings were all made from the peripheral cut end of the vagal nerve as previously described (Uneyama et al. 2002; Uneyama et al. 2006). Thus, multiunit afferent recordings were obtained from the ventral gastric vagal nerve. The abdominal wound was covered with saline-soaked gauze, and the rats were maintained at 37 °C with a heating pad (BWT-100, BRC, Nagoya, Japan).

Electrophysiological recordings

The electrode was connected to a head stage (JB-101 J, Nihon-Koden, Tokyo, Japan), and the signal was differentially amplified 10,000 times and filtered with a bandwidth of 150 Hz to 1 kHz (SEN-6000, Nihon-Koden, Tokyo, Japan). The output of the neural signal, together with the signal from a pressure transducer, was acquired by a power lab interface (Powerlab, ADInstrument) and viewed online with Macintosh-running Chart software. The nerve signal was digitally sampled at 4 kHz, which was sufficient to allow spike discrimination from background noise. Twenty minutes of baseline recording was performed after preparation of the nerve for signal stabilization. All nerve activity was analyzed after conversion of raw data to standard pulses and counted (5-s bin width) using an off-line software, spike histogram extension (SHE; ADInstrument) or Spike 2 (CED, UK), to distinguish discharges from background noise. Data were also recorded on tape for later analysis. Afferent nerve activities were characterized in terms of averaged discharge frequency (spikes/s, 5-s bin width). Baseline discharge was determined over a 30-s period just before drug administration, 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.

Chemical agents

Agents used in this experiment were serotonin (5-HT), sodium nitroprusside (SNP), *p*-chlorophenylalanine (PCPA), atropine, cimetidine, ketanserin (+) tartrate salt and S-nitroso-N-acetylpenicillamine (SNAP), all purchased from Sigma (St. Louis, USA). Cholecystokinin (CCK) and lorglumide were purchased from Peptide Institute (Osaka, JAPAN). Granisetron was from Chugai Pharmaceutical (Osaka, Japan). All chemicals were dissolved in saline or 0.5% carboxymetylcellulose sodium (CMC-Na) just before use in experiments.

Statistics

Data are expressed as mean ± SEM (n = 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. Analysis of variance was performed by using non-parametric tests such as the Wilcoxon signed-rank test or the Kruskal–Wallis test, as appropriate. A probability of $P<0.05$ was considered statistically significant.

Results

Effects of 5-HT and sodium nitroprusside (SNP) on gastric vagal afferents

Intravenous application of 5-HT and a NO donor, sodium nitroprusside (SNP), activated afferent nerves of the ventral gastric branches (Fig. 1**A**). The amplitudes of the spikes were not significantly different between 5-HT- and SNP-induced spikes (102.04 ± 2.79 μV in 10 μg/kg 5-HT; 102.95 ± 3.03 μV in the 300 μg/kg SNP). Maximal increases in discharge rates above basal firing induced by 10, 100 and 1000 μg/kg SNP were 2.0 ± 0.6, 7.6 ± 1.6, and 18.4 ± 3.0 spikes/s, respectively ($n=5$). The half-maximum effective dose (ED_{50}) of SNP for the afferent nerve activation was 168 ± 44 μg/kg. Minimal doses of SNP required for vagal afferent discharge and BP reduction were around 10 and 1 μg/kg, respectively. An increase in the afferent nerve discharge rate was observed by the intravenous application of another NO donor, SNAP (100 μg/kg) ($n=2$; data not shown).

A typical afferent discharge rate histogram and changes in arterial blood pressure (BP) in response to intravenous administration of 5-HT or SNP are shown in the Fig. 1**B**. Both 5-HT and SNP evoked afferent nerve responses but with markedly different latencies and time courses. 5-HT rapidly (<10 s) evoked a monophasic increase in afferent discharge during BP reduction, but SNP-induced afferent nerve responses were more complicated. SNP at 100 μg/kg (i.v.) gradually increased afferent nerve discharge rates after the blood pressure reduction reached maximal values. For example, the latency time for intravenously applied 10 μg/kg 5-HT was 5.7 ± 1.3 s for the BP response and 1.7 ± 10.4 s for afferent nerve activation ($n=4$). The latency time for intravenously applied SNP (100 μg/kg) was 6.9 ± 1.0 s for the BP response and 57.4 ± 8.5 s for the afferent nerve response ($n=4$). Moreover, SNP at 1000 μg/kg evoked a biphasic response with a first phase that reached maximal levels and a second phase that was delayed in onset but was maintained over a longer time period (>10 min). Intriguingly, the second phase of the afferent discharge was maintained even after the recovery of BP to the basal level (Fig. 1B right graph). Dose-dependent changes in latency times to 5-HT and SNP responses are summarized in Fig. 2. Latency time for the afferent nerve activation was shortened depending on the dose of SNP. Even at high doses of SNP (1000 μ/kg, i.v.), SNP required a 10-fold increase in time to evoke a neural response, as compared to the response to 10 μg/kg 5-HT.

Effects of 5-HT antagonists on SNP-induced vagal afferent activation

To identify the mucosal transmitter involved in SNP-induced afferent nerve activation, a pharmacological study was performed using various receptor antagonists. A muscarinic antagonist (atropine, 10 μg/kg, i.v.), a CCK-A antagonist (lorglumide, 1 mg/kg, i.v.), a 5-HT_2 antagonist (ketanserin, 100 μg/kg, i.v.) or an H_2 antagonist (cimetidine, 100 μg/kg, i.v.) did not block SNP-induced afferent discharge (each $n=2$, data not shown). However, intravenous administration of the 5-HT_3 antagonist granisetron strongly attenuated the SNP response. A typical effect of granisetron on the SNP response is shown in Fig. 3A. In this nerve bundle, administration of SNP increased the afferent discharge rate in a dose-dependent manner (10–3000 μg/kg, i.v.). The SNP-induced afferent response was attenuated completely immediately after granisetron (10 μg/kg, i.v.) injection. After granisetron treatment, 10 μg/kg 5-HT-induced responses were diminished, and re-administration of SNP did not evoke further responses. However, intravenous application of CCK-8 (300 ng/kg) markedly increased the afferent discharge, indicating that this nerve bundle selectively lost its 5-HT responsiveness. The effect of granisetron treatment on afferent discharge in response to 100 μg/kg SNP, 10 μg/kg 5-HT and 300 ng/kg CCK-8 are summarized

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