An enteric signal regulates putative gastrointestinal presympathetic vasomotor neurons in rats

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Sartor, Daniela M., Arthur Shulkes, and Anthony J. M. Verberne. An enteric signal regulates putative gastrointestinal presympathetic vasomotor neurons in rats. Am J Physiol Regul Integr Comp Physiol 290: R625–R633, 2006. First published October 20, 2005; doi:10.1152/ajpregu.00639.2005.—Ingestion of a meal results in gastrointestinal (GI) hyperemia and is associated with systemic and paracrine release of a number of peptide hormones, including cholecystokinin (CCK) and 5-hydroxytryptamine (5-HT). Systemic administration of CCK octapeptide inhibits a subset of presympathetic neurons of the rostroventrolateral medulla (RVLM) that may be responsible for driving the sympathetic vasomotor tone to the GI viscera. The aim of this study was to determine whether endogenous release of CCK and/or 5-HT also inhibits CCK-sensitive RVLM neurons. The effects of intraduodenal administration of the secretagogues sodium oleate (SO) and soybean trypsin inhibitor (SBTI) on circulating levels of CCK and 5-HT were examined. In separate experiments, the discharge rates of barosensitive, medullospinal, CCK-sensitive RVLM presympathetic vasomotor neurons were recorded after rapid intraduodenal infusion of SO-SBTI or water. Alternatively, animals were pretreated with the CCK receptor antagonists devazepide and lorglumide or the 5-HT3 antagonist MDL-72222 before SO-SBTI administration. Secretagogue infusion significantly increased the level of circulating CCK, but not 5-HT. SO-SBTI significantly decreased (58%) the neuronal firing rate of CCK-sensitive RVLM presynaptic neurons compared with water (5%). CCK1 receptor antagonists did not reverse SO-SBTI-induced neuronal inhibition (58%), whereas the 5-HT3 antagonist significantly attenuated the effect (22%). This study demonstrates a functional relation between a subset of RVLM presympathetic vasomotor neurons and meal-related signals arising from the GI tract. It is likely that endogenously released 5-HT acts in a paracrine fashion on GI 5-HT3 receptors to initiate reflex inhibition of these neurons, resulting in GI vasodilatation by withdrawal of sympathetic tone.

rostroventrolateral medulla; cholecystokinin; devazepide; MDL-72222; 5-hydroxytryptamine

GASTROINTESTINAL HYPEREMIA is a physiological consequence of food consumption. Gastrointestinal hormones such as cholecystokinin (CCK) have been implicated in gastrointestinal vasodilatation associated with postprandial increase in splanchnic blood flow. The involvement of the sympathetic vasomotor system in this response is poorly understood. We recently suggested that food-related signals may produce gastrointestinal vasodilatation via a reflex withdrawal of sympathetic vasomotor outflow (39, 45). In healthy individuals, the sympathetic nervous system compensates for postprandial splanchnic blood pooling by activating sympathetic vasoconstrictor drive to skeletal muscle resistance vessels in response to baroreceptor unloading. In patients with cardiovascular disease associated with diabetic neuropathy or autonomic dysfunction, poor baroreflex compensation for blood pooling in the splanchnic bed (9) may result in serious, and even life-threatening, consequences, including angina pectoris and stroke (17, 31).

Postprandial release of CCK in the process of food digestion has been well documented and leads to gallbladder contraction, satiety (35), pancreatic exocrine secretion, increased gastric motility, and gastrointestinal hyperemia (27). In rats, foods rich in protein (21, 29) and fats (20) are the most potent stimulants of CCK release (19). Fatty acids such as sodium oleate (SO) appear to function by direct stimulation of CCK-containing enteroendocrine cells of the gastrointestinal mucosa (3). Proteases enter the gut postprandially bind to trypsin to form an inactive complex, facilitating CCK release by removing the inhibitory influence of the protease on CCK-releasing peptides (16). Similarly, soybean trypsin inhibitor (SBTI) acts to prevent the digestion of trypsin-sensitive peptides by inhibiting trypsin activity (50), making it a potent stimulus for CCK release in rats (29).

It has been suggested that CCK and 5-hydroxytryptamine (5-HT) act synergistically to activate vagal afferents (24). Early in vivo studies supported a role for 5-HT in the vasodilator responses elicited by CCK, secretin, or mucosal irritation (12). Osmotic stimuli and the products of carbohydrate digestion stimulate the release of 5-HT from enterochromaffin cells lining the gastrointestinal mucosa (53).

Premotor sympathoeexcitatory (presympathetic) vasomotor neurons in the rostroventrolateral medulla (RVLM) are thought to have a pivotal role in blood pressure regulation (5, 41). Intravenous administration of CCK sulfated octapeptide (CCK-8) selectively inhibits splanchnic, but not lumbar, sympathetic nerve activity (39) and is also selective for a subset of barosensitive neurons in the RVLM (39, 40). Postprandially released CCK may act in a paracrine fashion on subdiaphragmatic vagal afferent fibers (38) within the wall of the gastrointestinal mucosa to indirectly inhibit RVLM presympathetic neurons. We hypothesized that these CCK-sensitive RVLM neurons may control gastrointestinal vascular resistance, and we demonstrated that the response to exogenous CCK was dependent on 5-HT3 receptors and central N-methyl-D-aspartate receptors (38, 47). However, the physiological significance of these findings was unclear, because it was not known whether endogenous release of CCK could mediate this effect via similar receptor interactions.

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The aim of this study was to investigate whether the physiological release of endogenous CCK and 5-HT, stimulated by intraduodenal secretagogue administration, could also inhibit the activity of CCK-sensitive RVLM neurons and to determine whether CCK₁ and/or 5-HT₃ receptors were involved.

**MATERIALS AND METHODS**

*Animals.* Male Sprague-Dawley rats (300–450 g body wt, n = 34) were obtained from the Animal Resources Centre (Perth, Western Australia). This study was approved by the Animal Ethics Committee of Austin Health and complied with principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

*Materials.* CCK-8 (sulfated form) was purchased from American Peptide (Sunnyvale, CA); phenylbiguanide (PBG) from Aldrich Chemical (Milwaukee, WI); lorglumide (sodium salt), SO (cis-9-octadecanocoic acid sodium salt), and SBTI from Sigma-Aldrich (St. Louis, MO); MDL-72222 from Tocris (Ellissville, MO); and devazepide (L-364718) from Merck Research Laboratories (Rahway, NJ).

*Animal preparation.* Arterial and venous cannulation of male Sprague-Dawley rats for the measurement of arterial blood pressure and for intravenous drug administration, respectively, was carried out as described previously (39, 40). Briefly, rats were anesthetized with halothane, tracheostomized, and ventilated with 100% O₂ containing 1.3–1.5% halothane (50–60 breaths/min, 1 ml/kg). This procedure was followed by cannulation of the left jugular vein and carotid artery for intravenous drug administration and measurement of arterial blood pressure, respectively. Adequacy of anesthetic depth was verified by the absence of a response to toe pinch or corneal probing. For duodenal administration of SO-SBTI or water, a single-lumen polyethylene catheter (0.58 mm ID, 0.96 mm OD; Critchley Electrical Products, Silverwater, NSW, Australia) was inserted into the duodenum in the aboral direction 0.5 cm distal to the pyloric sphincter and secured to the abdominal wall with a silk suture.

Extracellular recording of RVLM presympathetic vasomotor neurons was performed using glass microelectrodes (2 mm OD) filled with 2% Pontamine sky blue in 0.5 M sodium acetate. An intracellular amplifier in bridge mode (Intra 767, World Precision Instruments, Sarasota, FL) was used for recordings, and a window discriminator (Fintronics, Orange, CT) was used for signal amplification (400 Hz–4 kHz band pass); an oscilloscope and audio amplifier were used to monitor extracellularly recorded signals. The procedures used for location, identification, and extracellular single-unit recording of RVLM neurons have been described previously (39, 40). Before neuronal recording, an inflatable occlusive cuff was placed on the abdominal aorta for precisely controlled elevation of arterial blood pressure for baroreceptor stimulation. A bipolar electrode was placed in the dorsolateral funiculus of the spinal cord for antidromic activation of RVLM neurons. Another bipolar electrode was placed on the mandibular branch of the facial nerve, which was stimulated to create a field potential used to identify the location of RVLM neurons. After completion of all surgery, neuromuscular blockade was induced by administration of pancuronium (1–2 mg/kg iv).

The dose of CCK used to determine RVLM neuronal sensitivity to CCK was based on previous studies in our laboratory (39). Only barosensitive, medullospinal RVLM neurons with positive collision tests were used. These neurons were then tested for responsiveness to systemic administration of the 5-HT₃ agonist PBG (von Bezdol-Jarisch reflex; 5–10 µg/kg iv) and CCK-8 (4 µg/kg iv). The doses of CCK-8 and PBG were submaximal, as determined from previous studies (39, 46, 48). Only neurons that were inhibited by CCK were selected for this study, with the exception of those in the CCK-insensitive control group.

**Effect of SO-SBTI or water on neuronal firing rate.** A single neuron was studied in each experiment. Neuronal discharge was then monitored for up to 1 h after the rapid (>30 s) intraduodenal infusion of 1–2 ml of SO (dissolved in warm tap water, 100 mM final concentration) and SBTI (0.8% stock solution in distilled water, 0.2% final concentration). Two control groups were selected. One group, which consisted of barosensitive, spinally projecting RVLM neurons that were not CCK sensitive, was tested for response to intraduodenal infusion of SO-SBTI. In other control animals, the neuronal discharge of CCK-sensitive neurons was monitored after administration of 1–2 ml of tap water in place of SO-SBTI. In both groups, neuronal discharge was monitored for 1 h after duodenal infusion. As a standard procedure for all neuronal activity evaluated in this study, the percent decrease in discharge rate was calculated by dividing the difference between the mean basal firing rate and the mean firing rate during the period of sustained maximal inhibition (counted over a standard period of 70 s) by the mean basal firing rate and expressing this value as a percentage.

**Effect of CCK₁ receptor antagonism on SO-SBTI inhibition of RVLM neurons.** To determine whether postprandial release of CCK contributed to the inhibition of RVLM neuronal activity, a group of animals was pretreated with either of the CCK₁ receptor antagonists: devazepide (5 animals, 0.5–1.0 mg/kg iv) or lorglumide (1 animal, 10 mg/kg iv). Each antagonist was administered in separate experiments after location of a CCK-sensitive, RVLM presympathetic neuron. After administration of the antagonist and return of arterial pressure and/or neuronal firing rates to baseline levels (~20 min), the absence of a response to intravenously administered CCK was verified, and 1 ml of SO-SBTI was infused intraduodenally as described above. The neuronal discharge rate was then monitored for up to 1 h, and periods of inhibition were analyzed and compared with mean baseline levels.

**Effect of 5-HT₃ receptor antagonism on SO-SBTI inhibition of RVLM neurons.** To determine whether postprandial release of 5-HT contributed to the inhibition of RVLM neuronal activity, a group of animals was pretreated with the 5-HT₃ receptor antagonist MDL-72222 (0.5 mg/kg iv) following the procedure described above for the CCK₁ receptor antagonists.

**Circulating CCK and 5-HT levels after intraduodenal infusion of SO-SBTI.** In addition to the general procedures described above, arterial blood was collected from animals directly before and for 1 h after infusion of SO-SBTI into the proximal duodenum. In the case of circulating CCK levels, blood was collected 20, 40, and 60 min after secretagogue infusion. In a separate set of experiments, blood was collected for the determination of circulating 5-HT levels 15, 30, 45, and 60 min after infusion, because a smaller volume was required for assay. These experiments were not performed in conjunction with neuronal recording experiments, because removal of blood was likely to compromise the conditions required for stable extracellular recording of RVLM presympathetic neurons.

**Radioimmunoassay of plasma CCK.** Blood (900 µl) was collected into Eppendorf tubes containing 100 µl of heparin (1,000 U/ml). After collection, the blood samples were immediately placed on ice and centrifuged. Plasma was stored at −20°C until the time of assay. Plasma CCK concentration was measured in ethanol-extracted plasma as described previously (52). Briefly, 800 µl of absolute ethanol were added to 400 µl of plasma, and the mixture was centrifuged for removal of coagulated protein. The ethanol extracts were then divided into two 400-µl aliquots, evaporated to dryness, and stored at −20°C until time of assay. Antiserum 92128, which has negligible cross-reactivity with gastrins, was used in the assay. 125I-labeled Bolton-Hunter CCK-8 (Amersham International, Piscataway, NJ) was used as a tracer. Charcoal-stripped, ovine-extracted plasma tubes were used for the CCK-8 nonsulfated standard curve. The ID₅₀ was 3.5 fmol/ml, and the intra- and interassay coefficients of variation were 7 and <14%, respectively.

**Determination of whole blood 5-HT.** After collection of blood (500 µl) into Eppendorf tubes, platelet disruption was achieved by addition of 0.5 ml of ascorbic acid (9 mg/ml in distilled water) followed immediately by 0.2 ml of 4 M perchloric acid. Tubes were vortexed for 20 s and then centrifuged at 12,000 g for 10 min. Supernatants...
were stored at −20°C and assayed within 3 days of collection. Biochemical assays were performed using a reverse-phase high-pressure liquid chromatography machine fitted with a 15-cm Spherisorb 5-μm C18 column (Waters Australia, Rydalmere, NSW, Australia). An injector system (model LC10, Shimadzu Scientific Instruments Oceania, Mount Waverly, Victoria, Australia) was used for delivery of samples. Elution of the compound was monitored by a fluorescence detector (Kratos model FS970, Shimadzu Scientific Instruments Oceania; 200-nm excitation wavelength, Corning 7-60 emission filter). The mobile phase contained 0.1 M potassium dihydrogen phosphate, 0.4 M perchloric acid, and 0.1% sodium heptanesulfate in 10% acetonitrile (pH 4.0) and was run through the column at 2 ml/min. The detection limit was 1 ng/ml.

Data analysis and statistics. Arterial blood pressure, heart rate, extracellular action potentials, and stimulation pulses were recorded using a data acquisition system (Cambridge Electronic Design, Cambridge, UK) and Spike2 software. Values are means ± SE. Statistical analyses were performed using a one-way ANOVA followed by a Tukey-Kramer test using Instat version 3.06 (Graph Pad Software, San Diego, CA).

RESULTS

Electrophysiological characterization of RVLM presympathetic neurons and sensitivity to CCK. All units were spontaneously active and were silenced or considerably slowed by elevation of arterial blood pressure (Fig. 1A). In addition, all were transiently inhibited by administration of the 5-HT3 receptor agonist PBG (Fig. 1A). Apart from the CCK-insensitive controls, all neurons selected for this study were clearly inhibited by systemic administration of CCK-8 (Fig. 1A) and were spinally projecting (collision test positive; Fig. 1B).

Effect of water on RVLM neuronal firing rate. The fluctuations in neuronal firing rates after infusion of 1–2 ml of tap water into the duodenum ranged from 0 to 13% below baseline, with a mean decrease of 5 ± 2%: baseline firing rate = 22 ± 3 spikes/s and mean conduction velocity = 4.4 ± 0.6 m/s (n = 6). These small fluctuations in firing rate were short-lived, and all cells showing any sign of inhibition recovered during the course of the recording. Periods of inhibitory activity were analyzed and compared with baseline levels. These changes were not associated with any significant increases in arterial pressure and were usually accompanied by a decrease in arterial pressure (mean −7 ± 5 mmHg, n = 4) and heart rate (mean −8 ± 5 beats/min, n = 4). Similar blood pressure and heart rate fluctuations were observed in the two animals with no change in firing rate.

Effect of SO-SBTI on RVLM neuronal firing rate. Infusion of SO-SBTI into the proximal duodenum produced 29–100%
inhibition of RVLM neuronal spike discharge, with a mean decrease in firing rate of 58 ± 7% (mean conduction velocity = 3.3 ± 0.5 m/s, baseline firing rate = 11 ± 2 spikes/s, n = 10). Neuronal discharge rate was monitored for up to 1 h after infusion of SO-SBTI into the duodenum, with maximal inhibition of neuronal discharge at 22–50 min after infusion (mean 31 ± 3 min). Patterns in firing rate emerged whereby periods of sustained inhibition (>10 min) were gradually followed by periods of partial recovery. In two cases, cell firing was monitored for only 30 and 45 min because of instability and loss of recording. The inhibitory phase of these cells was nevertheless monitored for 10 and 25 min, respectively, and was sustained. In all other cases, once the cell showed signs of sustained inhibition, the firing rate did not return to pre-SO-SBTI levels during the course of the recording. Inhibition was invariably accompanied by a fall in arterial pressure and heart rate, with an average decrease of −22 ± 4 mmHg and −19 ± 4 beats/min (n = 10), respectively, confirming that the inhibition was not dependent on arterial pressure. Changes in arterial pressure and heart rate were not significantly different from the tap water control experiments (P > 0.05). Figure 2 depicts the effect of SO-SBTI on the neuronal discharge of an RVLM presympathetic vasomotor neuron. At 20 min after SO-SBTI infusion, firing rate was reduced by 70% (Fig. 2B). At 40 min after infusion, partial recovery of neuronal activity was evident (Fig. 2C). The blood pressure during maximal inhibition fell by 45 mmHg and the heart rate by 11 beats/min. The inhibition of RVLM neuronal firing rate produced by infusion of SO-SBTI into the duodenum was significantly different from that produced by infusion of water alone (Fig. 3; P < 0.001).

CCK₁ receptor antagonism. Pretreatment with devazepide or lorglumide before intraduodenal infusion of SO-SBTI did not attenuate the inhibitory effect of SO-SBTI on neuronal firing rate. Maximal inhibition (with respect to baseline levels) was 26–95% (mean 58 ± 12%, conduction velocity = 3.5 ± 0.3 m/s, baseline firing rate = 7 ± 1 spikes/s, n = 6) and occurred at 15–54 min (mean 41 ± 6 min) after SO-SBTI infusion. The inhibition observed in the group pretreated with CCK₁ receptor antagonists was not significantly different from that observed in the SO-SBTI-infused group without pretreatment (P > 0.05) but was significantly different from that observed in the water control group (P < 0.001; Fig. 3). After SO-SBTI infusion, sustained inhibition of neuronal firing rate was observed, with firing rate returning to baseline in only one case. Inhibition was invariably accompanied by a fall in arterial pressure and heart rate, with an average decrease of −27 ± 5

Fig. 2. Effect of sodium oleate (SO)-soybean trypsin inhibitor (SBTI) on discharge rate of an RVLM presympathetic vasomotor neuron. A: before infusion. B: 20 min after infusion; note 70% inhibition of cell firing rate. C: 40 min after infusion; note partial recovery of neuronal discharge.
The concentration of 5-HT in arterial blood was not significantly altered after SO-SBTI administration (Fig. 5). Baseline levels of 208.4 ± 54.5 ng/ml rose to a maximum of 244.0 ± 60.8 ng/ml (P > 0.05; n = 6) at 15 min after infusion and returned to baseline (205.2 ± 35.4 ng/ml) at 60 min after infusion.

DISCUSSION

We previously demonstrated that systemic administration of exogenous CCK inhibits the firing rate of a subpopulation of RVLM presympathetic (termed CCK-sensitive) neurons (39, 40). On the basis of these observations, we predicted that simulation of postprandial conditions by infusion of secretagogues into the duodenum would also inhibit CCK-sensitive RVLM presympathetic vasomotor neurons, and this hypothesis was tested in the present study. Before duodenal secretagogue infusion, the sensitivity of RVLM neurons to CCK was tested using intravenous administration of the peptide. In accordance with our previous findings, we found that CCK-sensitive neurons had axonal conduction velocity (mean 3.6 ± 1.4 m/s) consistent with lightly myelinated axons (40). Intraduodenal

Fig. 3. Inhibitory effects of intraduodenal administration of SO-SBTI on RVLM neuronal firing rate with or without antagonist pretreatment: comparison with water and non-CCK-sensitive controls. Duodenal infusion of SO-SBTI alone or with CCK1 receptor antagonist devazepide or lorglumide resulted in a decrease in RVLM neuronal firing rate that was significantly different (***P < 0.001) from water control. Effect of SO-SBTI on RVLM neuronal firing rate was significantly attenuated after 5-HT3 receptor antagonist MDL-72222 pretreatment compared with SO-SBTI alone (**P < 0.01) or CCK1 receptor antagonist pretreatment (***P < 0.001). Effect of SO-SBTI on non-CCK-sensitive neurons was not significantly different from water control or MDL-72222 pretreatment but was significantly different from SO-SBTI alone (***P < 0.001) or CCK1 receptor antagonist pretreatment (**P < 0.01).

Effect of SO/SBTI on whole blood 5-HT. The concentration of 5-HT in arterial blood was not significantly altered after SO-SBTI administration (Fig. 5). Baseline levels of 208.4 ± 54.5 ng/ml rose to a maximum of 244.0 ± 60.8 ng/ml (P > 0.05; n = 6) at 15 min after infusion and returned to baseline (205.2 ± 35.4 ng/ml) at 60 min after infusion.

Effect of SO-SBTI on non-CCK-sensitive controls. After intraduodenal administration of SO-SBTI, the magnitude of the inhibition observed in CCK-insensitive neurons was comparable to that observed in control and MDL-72222 pretreatment experiments (P > 0.05 in both cases), with fluctuations in neuronal firing rate of 0–22% (mean neuronal inhibition = 11 ± 4%, baseline firing rate = 15 ± 7 spikes/s, mean conduction velocity = 1.3 ± 0.4 m/s, n = 5; Fig. 3). Inhibitory periods were not associated with any significant increases in arterial pressure but, rather, with a decrease in blood pressure (mean −18 ± 6 mmHg, n = 4 excluding the experiment in which there was 0% decrease in firing rate, P > 0.05 compared with control) and heart rate (mean −21 ± 17 beats/min, n = 4). In all cases, the inhibition was transient and was followed by full recovery to preinfusion discharge rates.

Effect of SO-SBTI on circulating levels of CCK. Infusion of SO-SBTI into the duodenum invariably produced an elevation in circulating levels of CCK-8. Baseline levels of 8.0 ± 1.9 pM rose to maximal plasma concentrations of 20.1 ± 2.9 pM (P < 0.05, n = 5) at 20 min after infusion, amounting to an average 2.5-fold increase in circulating levels of CCK-8 (Fig. 4). Individual peak increases in CCK levels varied from 1.5- to 7-fold and occurred 20–60 min after intraduodenal infusion of SO-SBTI.
secretagogue administration inhibited the firing rate of CCK-sensitive RVLM presympathetic neurons, but not CCK-insensitive RVLM neurons. Intraduodenal administration of water in place of SO-SBTI had no significant effects on the firing rate of RVLM presympathetic vasomotor neurons. Surprisingly, the antagonist studies demonstrated the inhibitory effects of secretagogue administration on CCK-sensitive RVLM neurons to be independent of CCK₁ receptor activation but, instead, contingent on 5-HT₃ receptor activation.

Apart from CCK, several other gastrointestinal hormones are released during digestion, including somatostatin, leptin, gastrin, secretin, peptide YY, enteroglucagon, neutrotensin (13, 43), and 5-HT (32). Although the ability of CCK to augment gastrointestinal blood flow has been demonstrated (4, 10, 15, 39), other gastrointestinal hormones have been implicated in postprandial gastrointestinal hyperemia (4, 36). SO-SBTI may trigger the release of several hormones that may act on vagal afferents to inhibit RVLM presympathetic neuronal discharge. A possible candidate is 5-HT, given that it has been shown to activate cardiopulmonary and gastrointestinal vagal afferents via a 5-HT₃ receptor-dependent mechanism (2, 8). In support of this proposal, all RVLM neurons in this study, including those that were CCK sensitive, were also sensitive to systemic administration of the selective 5-HT₃ receptor agonist PBG.

5-HT may play a gastrointestinal vasodilator role in response to CCK (12). Our laboratory previously demonstrated that pretreatment with the selective 5-HT₃ receptor antagonist MDL-72222 attenuates the inhibitory effect of exogenously administered CCK on CCK-sensitive RVLM presympathetic neurons (38). It was hypothesized that the actions of CCK may have been partly dependent on 5-HT₃ receptor activation; i.e., CCK may release 5-HT, which acts on 5-HT₃ receptors to inhibit RVLM neuronal activity. In examining the effects of CCK on feeding, many have suggested that the actions of CCK are partially dependent on 5-HT₃ receptor activation (1, 6, 14). Furthermore, primary vagal afferent neurons are potently stimulated by endogenous 5-HT and CCK release (26, 53), and a subpopulation of nodose ganglion neurons possess high-affinity CCK₁ receptors and 5-HT₃ receptors (24).

The present study demonstrated that pretreatment with the 5-HT₃ receptor antagonist MDL-72222 significantly attenuated the inhibitory effect of SO-SBTI on RVLM neuronal firing, whereas pretreatment with the CCK₁ receptor antagonist devazepide had no effect. These results suggested that the secretagogue-induced inhibition of RVLM neurons was not CCK₁ receptor mediated but, instead, dependent on 5-HT₃ receptor activation. It is conceivable that SO-SBTI triggers an integrated response involving the release of CCK and other hormones, such as 5-HT, that result in vagal afferent activation and reflex inhibition of RVLM presympathetic neuronal discharge. However, because MDL-72222 readily crosses the blood-brain barrier, the possibility that it acts centrally cannot be discounted.

Our study corroborated the findings of previous investigations showing that infusion of SO or SBTI into the duodenum increases the levels of circulating CCK (42, 50), although the basal circulating CCK concentrations observed in the present study were approximately twofold higher than those reported elsewhere. Others reported peak levels after SBTI (28, 30) or SO (19) to be <11 pM compared with 20 pM observed in our laboratory. However, basal levels reported in other studies were lower than those observed in the present study (<1 pM compared with our 8 pM) as were the increases after secretagogue infusion (up to 16-fold compared with our 2.5-fold). These discrepancies may be due to several differences in the protocols employed by others, including the use of fasted, conscious animals. In support of these claims, Li and colleagues (20), who also performed studies on anesthetized animals and examined the effects of intraduodenal SO administration on endogenous CCK release, obtained basal and postsecretagogue CCK levels similar to ours (20).

In this study, RVLM presympathetic vasomotor neurons that were inhibited by intravenous administration of CCK-8 are referred to as CCK sensitive. The inhibitory effects of intravenously administered CCK-8 on the discharge of CCK-sensitive RVLM presympathetic neurons is blocked by the CCK₁ receptor antagonist devazepide (38; present study). We have proposed that CCK-sensitive RVLM presympathetic neurons may drive the sympathetic vasomotor outflow to the gastrointestinal vasculature and, therefore, may be involved in postprandial gastrointestinal hyperemia. Duodenal infusion of SO-SBTI reduced the discharge rate of CCK-sensitive, but not CCK-insensitive, RVLM presympathetic vasomotor neurons. This was a secretagogue-dependent mechanism, because infusion of water alone into the duodenum had no significant effect on neuronal discharge, and was independent of mean arterial pressure elevation, because no change or a modest decrease in mean arterial pressure was noted during the periods of maximal neuronal inhibition.

CCK₁ receptor antagonists did not reverse the inhibitory effects of SO-SBTI on RVLM presympathetic neuronal discharge, suggesting that this inhibition was not mediated by activation of the CCK₁ receptor. The dose of devazepide chosen in the present study blocked the inhibitory actions of exogenously administered CCK-8 on splanchnic sympathetic nerve activity and CCK-sensitive RVLM neurons (39; present study). Furthermore, many investigators have demonstrated that similar doses of devazepide are capable of reversing the effects of endogenously released CCK on pancreatic enzyme secretion (23, 33, 34), vagal afferent nerve discharge (7, 11, 18), gastric emptying (51), and food intake (37), suggesting that the dose chosen in our study is likely to have been adequate.

It is possible that, despite an elevation in circulating levels of CCK, its concentration may have been insufficient for activa-
tion of subdiaphragmatic vagal afferents. As a result, the inhibitory effects of SO-SBTI on RVLM neuronal discharge were not blocked by devazepide. This prompted the conclusion that secretagogue-induced inhibition of CCK-sensitive RVLM neurons is not dependent on CCK1 receptor activation. Instead, the concomitant release of 5-HT from enterochromaffin cells after secretagogue infusion appears to be of greater importance.

Rat chow stimulates pancreatic protein secretion via a CCK-independent mechanism involving 5-HT release from enterochromaffin cells (22). The normal rat diet consists of 4–5% fat (42) and includes high-protein soybean meal (Barastoc G.R.2 rat and mouse feed content; Ridley Agriproducts, Pakenham, Victoria, Australia); SO is a digestion product of fat (49). The CCK secretagogues SO and SBTI used in our study are possible substitutes for the fat and protein content of rat chow, respectively, and may have stimulated the release of 5-HT from enterochromaffin cells without elevating its circulating levels. Li and colleagues (22) demonstrated that luminal non-CCK-mediated pancreatic secretion was eliminated after administration of the 5-HT synthesis inhibitor p-chlorophenylalanine. In another study (25), they also showed that maltose and hypertonic saline increased the intraluminal concentrations of 5-HT without affecting its circulating levels and that the monoamine acts in a paracrine fashion to induce pancreatic secretion by stimulating 5-HT3 receptors on gastrointestinal vagal afferents. Similarly, the release of 5-HT from enterochromaffin cells in response to SO-SBTI in the present study may have stimulated 5-HT3 receptors located on vagal sensory fibers in a paracrine fashion to produce a reflex decrease in sympathetic vasomotor tone. This is supported by the results demonstrating an attenuation of the SO-SBTI-induced inhibition of RVLM neuronal discharge rate after 5-HT3 receptor blockade.

The absence of a significant increase in arterial 5-HT concentration after SO-SBTI infusion into the duodenum is most probably due to its rapid and complete clearance as it passes through the pulmonary circulation (44). This also supports the notion that the inhibitory effects of 5-HT on RVLM neuronal discharge are likely to be mediated by luminal release and....
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paracrine activation of 5-HT₃ receptors on gastrointestinal vagal afferents, and not by activation of cardiopulmonary 5-HT₃ vagal afferents and the von Bezold-Jarisch reflex. Furthermore, others have demonstrated that nodose neuronal responses to digestion products and luminal osmolarity are dependent on the endogenous release of 5-HT from enterochromaffin cells lining the gastrointestinal mucosa (53). These responses were reported to occur within 5 min of continuous infusion of various test solutions. Although we have reported the maximal effects of secretagogue infusion on CCK-sensitive RVLM sympathetic neurons to occur (on average) at 31 min after infusion, neurons demonstrated signs of inhibition as early as 5 min (mean 10 ± 1, n = 10) after infusion. The reflex effects of intraduodenal nutrient infusion on RVLM neurons in our experiments may be slightly delayed compared with other preparations for several reasons, including administration of pancuronium, position of the gut due to positioning the rat in a stereotaxic frame, and bolus vs. continuous infusion of nutrients into the gut.

Our results demonstrate that a subset of RVLM presympathetic neurons are sensitive to systemic administration of CCK-8 and PBG (Fig. 6). These neurons may drive presympathetic vasomotor outflow to the gastrointestinal vasculature to produce gastrointestinal vasodilatation by an indirect intramedullary pathway similar to the von Bezold-Jarisch reflex. This reflex possibly functions via a trisynaptic medullary pathway, whereby neurons in the nucleus of the solitary tract, which receive excitatory vagal afferent inputs, activate inhibitory GABAergic caudal ventrolateral medullary neurons. These, in turn, inhibit RVLM presympathetic neurons (45), resulting in the reflex withdrawal of sympathetic vasomotor tone and subsequent vasodilatation (Fig. 6). We have found that inhibition of caudal ventrolateral medullary neurons by local microinjection of the GABA₄ receptor agonist muscimol abolishes baroreflex-mediated sympathoinhibition as well as CCK-induced inhibition of splanchic nerve discharge (unpublished observations).

Postprandial stimuli inhibited CCK-sensitive RVLM presympathetic neurons but not CCK-insensitive neurons. Devacipide failed to reverse the secretagogue-induced inhibition of CCK-sensitive RVLM presympathetic neurons, suggesting that the inhibition was independent of CCK₁ receptor activation. This implies that these neurons are not inhibited by the postprandial release of CCK, but by some other hormone released in response to SO-SBTI. Because these neurons are also sensitive to exogenous administration of PBG, it seems plausible that 5-HT released postprandially may act in a paracrine fashion (25, 53) on subdiaphragmatic vagal afferent neurons to produce gastrointestinal vasodilatation (Fig. 6).

The observation that only a subset of RVLM presympathetic neurons is CCK sensitive may be used to distinguish them from other RVLM presympathetic vasomotor neurons to elucidate their role in gastrointestinal circulatory control. This is the first study to describe a relation between a group of physiologically identified presympathetic vasomotor neurons of the RVLM and an enteric signal. Although these neurons are CCK sensitive, their physiologic role in gastrointestinal postprandial hyperemia seems to be independent of CCK₁ receptor activation. Instead, their contribution to gastrointestinal vascular perfusion may depend on activation of 5-HT₃ receptors, which are probably located on CCK-sensitive subdiaphragmatic vagal afferents, to elicit a novel gut-brain reflex. Further examination of these neurons and their role in gastrointestinal perfusion may lead to improved drug therapies for the treatment of postprandial gastrointestinal hyperemia, which can be symptomatic in susceptible individuals with compromised reflex sympathetic vasomotor control.

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REFERENCES

ENTERIC MODULATION OF PRESYMPATHETIC VASOMOTOR NEURONS


