Electrical physiological evidence for high- and low-affinity vagal CCK-A receptors

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Li, Ying, Jinxia Zhu, and Chung Owyang. Electrical physiological evidence for high- and low-affinity CCK-A receptors. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G469–G477, 1999.—We have demonstrated that under physiological conditions CCK acts through vagal high-affinity CCK-A receptors to mediate pancreatic secretion. In this study, we evaluated the vagal afferent response to endogenous CCK in rats and defined the CCK-receptor affinity states and the vagal-receptive field responsive to CCK stimulation using electrophysiological studies. Experiments were performed on anesthetized rats prepared with bile-pancreatic fistula. Plasma CCK levels were elevated by diverting bile-pancreatic juice (BPJ). The single-unit discharge of sensory neurons supplying the gastrointestinal tract was recorded from the nodose ganglia. All units studied were either silent or they had a very low resting discharge frequency. Thirty-two single units were studied extensively; seven were shown to be stimulated by diversion of BPJ (2.6 ± 2 impulses/min at basal to 40 ± 12 impulses/min after diversion). Acute subdiaphragmatic vagotomy or perivaginal capsaicin treatment abolished the response. The CCK-A-receptor antagonist CR-1409, but not the CCK-B antagonist L-365260, blocked the vagal response to endogenous CCK stimulation. Infusion of low-affinity CCK-receptor antagonist CCK-J-MV-180 completely blocked the vagal afferent response to the diversion of BPJ in three of seven rats tested but had no effect on the response in the remaining four. In a separate study, we demonstrated that gastric, celiac, or hepatic branch vagotomy abolished the response in different subgroups of neurons. In conclusion, under physiological conditions, CCK acts on both high- and low-affinity CCK-A receptors present on distinct vagal afferent fibers. The vagal CCK-receptor field includes the regions innervated by the gastric, celiac, and hepatic vagal branches. This study provides electrophysiological evidence that vagal CCK receptors are present on the vagal gastric, celiac, and hepatic branches and may occur in high- and low-affinity states.

nodose ganglia; cholecystokinin-J MV-180; selective branch vagotomy

CCK receptors have been detected in the rat vagus nerve using in vitro receptor autoradiography (51). Nerve ligation experiments demonstrate that these receptors are transported toward the peripheral nerve endings from the nodose ganglia (31, 51). Electrophysiological studies indicate that exogenous CCK influences the discharge of vagal afferent fibers or the brain stem neurons (7–9, 34, 35). Currently there is controversy regarding the specific vagal targets for CCK (7, 9, 34, 35). Little is known about the electrophysiological behavior of the nodose ganglia in response to physiological concentrations of CCK, and the vagal receptive field responsive to endogenous CCK stimulation has not been clearly defined.

CCK receptors in the peripheral vagal afferent fibers may mediate several digestive functions. There is evidence that CCK stimulates vagal afferent fibers, altering the vagovagal reflex of proximal gastric motility and emptying (36). Perivaginal capsaicin applications significantly reduced the inhibitory action of CCK (36). The afferent fibers of the abdominal vagus nerve also appear to mediate CCK elicited satiety signals to the area postrema nucleus in the dorsal hind brain (12, 44). In pancreatic acini, CCK receptors exist in both high- and low-affinity states (38, 48). CCK dose-response studies using pancreatic acini typically reveal a biphasic dose-response relationship: stimulation of amylase release at low CCK concentrations and inhibition at supramaximal concentrations (38, 48). We recently demonstrated that under physiological conditions CCK acts through high-affinity CCK-A receptors on the vagal afferent fibers to mediate pancreatic secretion (21–23). Other investigators have reported that activation of gastric mechanosensitive vagal afferent fibers (40) and suppression of food intake (50) by CCK are mediated by vagal low-affinity CCK-A receptors in rats. However, electrophysiological evidence for vagal high- and low-affinity CCK receptors is lacking.

In this study we investigated the vagal afferent response to endogenous CCK by performing electrophysiological studies in the rat. The discharges of sensory neurons supplying the gastrointestinal tract were recorded from the nodose ganglia. Plasma CCK levels were elevated by diversion of bile-pancreatic juice (BPJ), a method known to increase endogenous CCK secretion. Chemical ablation studies were performed to identify vagal afferent fibers responsible for nodose activities in response to CCK stimulation. To evaluate the vagal target sites responsible for vagal afferent responses to endogenous CCK, we performed transections of distinct branches of the vagus nerve. We used CCK-J-MV-180, which acts as an agonist on high-affinity receptors and as an antagonist on low-affinity CCK receptors, to identify the vagal CCK-receptor affinity states in the mediation of the vagal afferent response to the diversion of BPJ.

METHODS
Materials
The following materials were purchased: soybean trypsin inhibitor (SBTI, type II-s) and capsaicin from Sigma Chemi-
VAGAL AFFERENT RESPONSES TO ENDOGENOUS CCK

Animal Preparation

Experiments were performed on adult male Sprague-Dawley rats weighing 270–350 g. The animals were anesthetized with a mixture of xylazine and ketamine (13–22 and 87–140 mg/kg body wt, respectively). Supplemental doses of the anesthetic agents were administered as needed to maintain a deep level of anesthesia and muscle relaxation. The level of anesthesia was determined by monitoring the heart rate (180 beats/min) and demonstrated that the rats exhibited no reflex response to tail pinching throughout the experiments under this condition. The doses of anesthetic agents used to maintain a deep level of anesthesia also markedly depressed normal respiration, which become shallow, irregular, and less than 50 breaths/min. Therefore, a tracheotomy was performed, and a tube was inserted into the trachea to permit artificial ventilation with room air (75–85 strokes/min, 3.5–4.0 cm³ tidal volume). A midline abdominal incision exposed the abdominal vagus, stomach, and duodenum. To stimulate the subdiaphragmatic vagal trunks, one pair of Teflon-coated, pure-gold wire, stimulating electrodes (outside diameter, 76 µm) were placed around the anterior and posterior trunks of the subdiaphragmatic vagal nerve, ~2–3 cm above the gastroesophageal junction and above the accessory and celiac branches of the vagus nerve. The stimulating electrodes were loosely sutured to the esophagus to limit their displacement. A polyethylene catheter (PE-10) was inserted into the common bile pancreatic duct at the ampulla to collect BPJ. A second catheter (PE-50) was placed in the duodenum, slightly above the sphincter of Oddi, for intestinal perfusion of BPJ and STBI. A third catheter was inserted into the ileum for the return of the BPJ during diversion. A polyethylene catheter connected to a syringe-driven pump was placed in the external jugular vein for intravenous infusion. The abdomen was closed, and BPJ was returned to the duodenum every 15 min. All experimental procedures have been approved by the University of Michigan Animal Use Committee.

Unit Activity Recording of Neurons From Nodose Ganglion

The rats were placed in a small Kopf animal stereotaxic frame. Their body temperature was maintained by a special heating bag. The right nodose ganglia was exposed by a short dorsal approach. Under an operating microscope, the ganglia sheath was removed and separated from the adjacent cervical sympathetic trunk and carotid artery. The discharge of sensory neurons supplying the gastrointestinal tract was recorded from the nodose ganglia by means of extracellular glass-coated tungsten microelectrodes (DC resistance of 3–5 MΩ). The recording microelectrode was driven progressively into the ganglia while the vagus nerve was stimulated electrically. Search stimuli consisted of a positive or negative rectangular pulse (0.5-ms duration, 3–8 V, 1 Hz isolated pulse stimulator model 2100, with biphasic pulse sign; A-M Systems, Everett, WA). An electrode was placed on a skin incision near the recording electrode as a reference electrode. Only the gastrointestinal C-fibers were recorded. C-fibers were identified in terms of latency (60–80 ms), conduction distance (between stimulating electrode and nodose ganglion, 0.06 m), and conduction velocity (<1.0 m/s) in response to electrical stimulation of the abdominal vagus (Fig 1). Vagal afferent unit discharges were amplified by an A-M Systems Neuroprobe Amplifier after high-input impedance preamplification and were monitored with an oscilloscope and audio monitor. The single-unit discharges were extracted from the multiunit signal on-line using a window discriminator. The upper and lower limits of the window discriminator were set until the range encompassed the activity of only one single unit (see Figs. 3, 5, and 7). The discharges were displayed and stored on a PC with a 166 MHz pentium processor computer using Axotape software (Axon Instruments). The basic discharge was monitored for 30 min to confirm the stability of the basal firing frequency. Movement artifacts were not observed because of the deep anesthesia and because the proximity of the reference electrode to the recording electrode prevented electrocardiogram and possible electromyogram interferences. To ensure that the same units were monitored consistently, special attention was given to the firing pattern of each unit, as well as to the amplitude and waveform of each spike. During the study and at its termination, the firing pattern of the spikes was reevaluated to ensure that it was identical to the original spike pattern (Fig 2).

Experiment Design

Vagal afferent response to diversion of BPJ. After a 30-min recording of the basal spontaneous activities, BPJ was diverted and returned to the ileum. The duodenum was perfused with saline containing STBI, 10 mg/kg at 1 ml for 5 min, to remove any protease activity from the proximal intestine.
by pulling the sutures out. A 60-min diversion of BPJ, vagal transections were performed, and 3-0-silk sutures were passed around the nerve trunks. After a 60 min, the subdiaphragmatic vagal trunks were carefully dissected, and the anterior and posterior trunks of the vagal nerves were carefully dissected, and 3-0-silk sutures were passed around the nerve trunks. After a 60-min diversion of BPJ, vagal transections were performed by pulling the sutures out.

Perivagal application of capsaicin. To investigate the role of the vagal afferent pathway in the mediation of CCK action, the effects of perivagal capsaicin application were examined. After anesthesia, the abdominal vagal trunks were exposed. A small piece of gauze soaked in 1% capsaicin solution (0.1 ml/rat) was left on the vagal trunks for 30 min. Solutions of vehicle (Tween 80 and olive oil) alone were applied to the vagus in the control rats. Vagal afferent response studies as described previously were performed 7 days after surgery.

Effects of CCK-A-receptor antagonist CR-1409 and CCK-B-receptor antagonist L-365260 on vagal afferent response to diversion of BPJ. Previous studies have shown that both CCK-A and CCK-B binding sites are transported toward the periphery of the rat vagus nerve (17). To investigate if the vagal afferent response evoked by diversion of BPJ involves the CCK-A receptor or the CCK-B receptor, we examined the effects of the CCK-A-receptor antagonist CR-1409 (10 mg/kg intravenous bolus injection, dissolved in 0.005 N NaOH) and the CCK-B-receptor antagonist L-365260 (3.5 μmol·kg⁻¹·h⁻¹), dissolved in methyl sulfoxide, Tween 80, and sterile 0.15 M NaCl, 8:1:1 vol/vol/vol). It has been shown that in the anesthetized rat, the peptide antagonist CR-1409 abolished cerulein-stimulated pancreatic secretion in a dose-dependent manner. At a dose of 10 mg/kg, CR-1409 abolished pancreatic response to a near-maximum dose of cerulein (32). In the anesthetized rat and cat, L-365260 dose-dependently inhibited pentagastrin-induced stimulation of gastric acid secretion, with an ID₅₀ of 2.5 μmol·kg⁻¹·h⁻¹ (3). This dose of L-365260 produced maximal inhibitory effect on the activation of rat stomach histidine decarboxylase by gastrin (10) but did not affect CCK-8-evoked gastric emptying. Vagal unitary responses to division of BPJ were monitored as described previously. The CCK-A- and CCK-B-receptor antagonists were administered 60 min after diversion of BPJ.

Effects of selective branch vagotomy on vagal afferent response to division of BPJ. To examine the role of distinct branches of the subdiaphragmatic vagus nerve in mediating the vagal unitary responses to division of BPJ, we performed distinct branch vagotomies. The two trunks of the abdominal vagus and the five major branches were identified using the method described by Prechtl and Powley (33). Both ventral and dorsal gastric vagal trunks were exposed by teasing them gently apart from the descending esophagus. Silk sutures (3-0) were passed around each gastric branch. Vagal gastric branches were cut by pulling the sutures out after a 60-min diversion of BPJ. In a separate group of rats, the hepatic branch was identified by gentle retraction of the stomach, and the hepatogastric omentum was splayed to reveal the anterior vagal trunk. Haptic branch vagotomy was performed using the same method as described previously. The accessory celiac branch divides from the anterior vagal trunk immediately after the hepatic branch ramifications. At about the midpoint of the esophagus, the celiac branches were separated from the main posterior vagal trunk. Both accessory and celiac branches were transected using the previously described method.

CCK-J MV-180 studies. CCK-J MV-180 is believed to interact with both classes of pancreatic CCK receptors, acting as an agonist at high-affinity states and an antagonist at low-affinity states (39, 46). To identify the vagal CCK-A-receptor affinity state that mediates CCK-evoked vagal afferent activities, we studied the effect of CCK-J MV-180 on the nodose ganglia responses after the diversion of BPJ. We have previously shown that intravenous infusion of CCK-J MV-180 produced a dose-dependent increase in pancreatic protein secretion via a capsaicin-sensitive vagal afferent pathway. CCK-J MV-180 at doses of 22 and 44 μg·kg⁻¹·h⁻¹ stimulated protein secretion over basal by 48 and 82%, respectively, in anesthetized rat (21) and by 64 and 120% in conscious rat (24). These doses stimulated pancreatic secretion to a degree similar to that observed with a physiological dose of CCK-8 (22, 24). On the other hand, CCK-J MV-180 has been shown to block suppression of food intake by CCK, which is mediated...
by vagal low affinity CCK-A receptors in rats (50). CCK-J MV-180 was infused at a dose of 44 µg·kg$^{-1}$·h$^{-1}$ after a 60-min diversion of BPJ, and it was administered throughout the remainder of the experiment.

Neurophysiological analysis. Neuronal responses were examined using the Datapac software system (Run Technologies, Laguna Niguel, CA). Prestimulus time histograms were constructed for the 30-min period before the intestinal infusion of SBTI and the diversion of BPJ. Time histograms were constructed for the 90- to 180-min period after the diversion of BPJ. During this period, the effects of various treatments, such as the return of BPJ, vagotomy, and the administration of CCK-receptor antagonists, were examined. The mean activity during the diversion of BPJ was compared with the prediversion activity and the activity after surgical and pharmacological interventions.

Statistical analysis. Results were expressed as means ± SE. Multivariant ANOVA was used to evaluate the effects of the repeated measurements over time, the effects of treatment, and the interaction between the two. Significance was accepted at the 5% level.

RESULTS

Effects of Diversion of Bile Pancreatic Juice on Vagal Afferent Discharge

Previously, we demonstrated that diversion of BPJ resulted in a fourfold increase in pancreatic output, peaking within 45 min, and plasma CCK levels increased from a basal of 0.7 ± 0.4 to 9.2 ± 1.8 pmol/l (20). In this study, following nodose recording, blood samples were obtained by cardiac puncture and plasma CCK levels were measured in four rats after a 60-min diversion of BPJ. We confirmed that a similar increase in plasma CCK levels was observed under this experimental condition (data not shown). The neuronal response of the nodose ganglia to the diversion of BPJ was evaluated in 32 single-unit recordings. Under basal conditions, all units studied were either silent or had a very low resting discharge frequency. Duodenal infusion of SBTI and diversion of BPJ resulted in a marked increase in neuronal discharge in 7 of 32 neurons tested. The discharge frequency increased from 2.6 ± 2 impulses/min to a peak of 40 ± 12 impulses/min after a 60-min diversion of BPJ (Fig. 3). It declined rapidly when BPJ was returned to the duodenum and gradually returned to basal levels 24 min after BPJ return (Figs. 3 and 4).

Subdiaphragmatic Vagotomy

Data were collected from 4 of 13 unitary recordings in 13 rats. After acute vagotomy, all units previously activated by electrical stimulation of the vagus nerve were retested to confirm that identical units were monitored. Acute vagotomy abolished the unitary re-
responses to the diversion of BPJ (Fig. 5). The discharge frequencies returned to basal levels within 2 min.

Perivagal Application of Capsaicin

Ten rats received a perivagal application of capsaicin and ten rats were pretreated with a vehicle solution 7 days before the experiment. In the vehicle-treated rats, 4 of 10 units tested increased their discharge in response to the diversion of BPJ from a basal of 1.3 ± 1 impulses/min to a peak of 51 ± 6 impulses/min after a 60-min diversion of BPJ. In the perivagal capsaicin-treated rats, all 10 units studied responded to electrical stimulation of the subdiaphragmatic vagus, but none responded to the diversion of BPJ (Fig. 6).

Effects of Administration of CCK-A and CCK-B-Receptor Antagonists

After a 60-min diversion of BPJ, administration of the CCK-A-receptor antagonist CR-1409 completely abolished the nodose afferent firing in three units. In contrast, the CCK-B-receptor antagonist L-365260 did not affect the firing rate in response to diversion of BPJ in the other four neurons (Fig. 7).

Effects of Distinct Branch Vagotomies

Eleven nodose neurons from 10 rats, which showed an increase of the discharge frequency after a 60-min diversion of BPJ, were studied. Acute gastric branch vagotomy resulted in 94% inhibition of the response in 4 of 11 neurons tested, but the responses of the other seven neurons were unaffected. Combining gastric and celiac branch vagotomies produced 96% inhibition of the response in three of these seven units; the response of the other four neurons remained unaffected. Hepatic branch vagotomy abolished the response in the four remaining neurons (Fig. 8).

CCK-J MV-180

After a 60-min diversion of BPJ, eight neurons that responded to the diversion of BPJ were tested. Administration of CCK-J MV-180 produced a marked inhibition in three of eight neurons (Fig. 9). This treatment, however, did not affect the neural responses to the diversion of BPJ in the other five neurons (Fig. 9). These observations suggested that endogenous CCK acts on both high- and low-affinity CCK-A receptors.

DISCUSSION

Vagal afferent fibers represent a major target for CCK, mediating a number of digestive functions (1, 22, 36, 44). However, the specific vagal target for exogenous infused CCK remains poorly defined. CCK has been shown to stimulate gastric muscle mechanoreceptors in the rat (7, 9, 41). Inhibition of gastric mechanoreceptor activity by CCK-8 has also been reported, whereas mucosal afferents in the gastroduodenal region appear to be stimulated by CCK in the ferret (7) and rat (37). Most of these studies used exogenous
bolus doses of CCK, and electrophysiological recordings were obtained by using the single-fiber recording technique, in which nerve bundles and filaments were teased from the main trunk. Off-line computer analysis of the afferent activity in the mesenteric nerve bundle has been demonstrated (37). Mei (27, 28) used extracellular microelectrode recordings from cell bodies in the nodose ganglia of cats. He showed (27) that extracellular recordings from ganglion cells are sensitive and can readily yield information about unmyelinated fibers, which are difficult to tease from the fine visceral nerves. The effect of bolus doses of CCK on vagal afferent fibers has been widely examined, but there are no reports of the electrophysiological characteristics of the nodose ganglia, the first order of sensory neuron, in response to endogenously released CCK under physiological conditions. Using the nodose ganglia recording technique in the anesthetized rat, we demonstrated the exquisite sensitivity of vagal sensory neurons to endogenous CCK. Under physiological conditions, CCK acts on both high- and low-affinity CCK-A receptors present on distinct vagal afferent fibers. The vagal CCK-receptor field includes the regions innervated by gastric, celiac, and hepatic vagal branches.

Diversion of BPJ from the duodenum stimulates CCK release and pancreatic enzyme secretion (23, 25). The increase in plasma CCK levels after the diversion of pancreatic juice is mediated by a trypsin-sensitive

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**Fig. 7. Effects of CCK-A- and CCK-B-receptor antagonists on vagal afferent response to diversion of BPJ.** Left: histogram representation of complete course of recording (firing rate count vs. time). Right: original unit action potential records selected from different periods of study. CCK-A-receptor antagonist CR-1409 abolished neural discharge evoked by diversion of BPJ (top). CCK-B-receptor antagonist L-365260 had no effect on firing rate in response to BPJ diversion (bottom), suggesting that response of neurons from nodose ganglia to endogenous CCK is mediated by vagal CCK-A receptor.

**Fig. 8. Effects of transection of gastric, celiac, and hepatic branch of vagus nerve on vagal afferent responses to BPJ diversion.** Eleven neurons from 10 rats, which demonstrated an increase in discharge frequency after 60-min period of diversion of BPJ, were studied. Acute transection of gastric branch abolished neuronal discharge in 4 of 11 neurons tested. In contrast, responses of the other 7 neurons were unaffected (left). Combining gastric and celiac branch vagotomy abolished response in 3 of 7 units tested, but the response of the other 4 neurons remained unaffected (middle). Hepatic branch vagotomy abolished response in remaining 4 neurons (right). These observations suggest that gastric, celiac, and hepatic branches all contain CCK-A receptors capable of responding to endogenous CCK after BPJ diversion. *P < 0.05.
different branches has been shown to mediate different nucleus of the solitary tract (2). Activation of the different branches project to different regions of the esophageal level, five distinct primary branches into the dorsal and ventral trunks. At the subdiaphragmatic level, five distinct primary branches. Although this study did not specifically address where these vagal afferent fibers terminate, it clearly indicated that the vagal CCK-receptive field includes all of the gastric, celiac, and hepatic branches. The electrophysiological evidence is compatible with the reports of previous functional studies (4, 18, 19, 21–24, 42, 49) and supports the concept that vagal CCK receptors in different sites may mediate different digestive and metabolic functions. Previous studies demonstrated that the gastric vagus contributes to the control of the emptying of nutrient and nonnutrient solutions, as well as to the inhibition of emptying produced by exogenous CCK (42). Vagal celiac branches mediate the suppression of feeding produced by intestinal infusion of nutrients (49). The major water absorption-signaling pathways project through the hepatic vagal branch (4), and CCK inhibits real and sham feeding, even after transection of all abdominal vagal branches except the hepatic branch (18, 19). We have demonstrated that gastroduodenal, but not jejunal, mucosal application of capsaicin abolishes pancreatic exocrine secretion in response to physiological doses of CCK (22). On the basis of the efferent vagal innervation pattern in the rat, the two celiac branches appear to innervate the jejunum, ileum, and the entire colon exclusively. The hepatic branch runs in the lesser omentum to the liver, bile duct, and part of the duodenum, with minor projections to the distal antral stomach and the intestine. The two gastric branches innervate the entire stomach and most of the proximal duodenum (5).

In this study, we showed that transection of the gastric vagal branches inhibited vagal afferent firing in 4 of 11 neurons tested, indicating that the gastric branch plays a significant, but not exclusive, role in the mediation of endogenous CCK-evoked vagal afferent activities. We also demonstrated that combined gastric and celiac branch vagotomy eliminated nodose discharge in response to diversion of BPJ in three of seven units, suggesting that the celiac branch also plays a role in the effects of endogenous CCK. A role for the hepatic vagal branch was suggested by the continued nodose neuronal discharge after transection of all abdominal vagal branches, except the hepatic branch, in four of seven units tested; the response was completely abolished after combined transection of all the vagal branches. Although this study did not specifically address under which conditions this would occur, it is possible that the response would be abolished after combined transection of all the vagal branches. However, this study did not specifically address where these afferent fibers terminate, it clearly indicated that the vagal CCK-receptive field includes all of the gastric, celiac, and hepatic branches. The electrophysiological evidence is compatible with the reports of previous functional studies (4, 18, 19, 21–24, 42, 49) and supports the concept that vagal CCK receptors in different sites may mediate different digestive and metabolic functions. Previous studies demonstrated that the gastric vagus contributes to the control of the emptying of nutrient and nonnutrient solutions, as well as to the inhibition of emptying produced by exogenous CCK (42). Vagal celiac branches mediate the suppression of feeding produced by intestinal infusion of nutrients (49). The major water absorption-signaling pathways project through the hepatic vagal branch (4), and CCK inhibits real and sham feeding, even after transection of all abdominal vagal branches except the hepatic branch (18, 19). We have demonstrated that gastroduodenal, but not jejunal, mucosal application of capsaicin abolishes pancreatic exocrine secretion in response to physiological doses of CCK (22). On the basis of the efferent vagal innervation pattern in the rat, the two celiac branches appear to innervate the jejunum, ileum, and the entire colon exclusively. The hepatic branch runs in the lesser omentum to the liver, bile duct, and part of the duodenum, with minor projections to the distal antral stomach and the intestine. The two gastric branches innervate the entire stomach and most of the proximal duodenum (5).

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affinity CCK-A receptors. In contrast to its effect on satiety, we demonstrated that intravenous infusion of CCK-J MV-180 at dose of 44 µg·kg$^{-1}$.h$^{-1}$, which stimulated pancreatic secretion to a degree similar to that observed with physiological dose of CCK-8 (22, 24), did not inhibit but rather enhanced the pancreatic responses to exogenous CCK and endogenous CCK, suggesting that under physiological conditions, CCK acts through high-affinity CCK-A receptors to mediate pancreatic protein secretion (20). In this study, we showed that administration of CCK-J MV-180 at a dose of 44 µg·kg$^{-1}$.h$^{-1}$ inhibited nodose neuronal responses to diversion of BPJ in three of eight neurons, but did not affect the responses in the other five neurons. Thus we provided electrophysiological evidence that both low- and high-affinity CCK receptors are present in the vagal nodose ganglia. Activation of these CCK receptors in different affinity states may mediate different digestive and metabolic functions.

Vagal afferent endings also respond to tension generated during stretch and contraction of the gastrointestinal tract (9, 40). In addition, CCK binding sites have been located on the circular muscle layer of the pyloric sphincter using autoradiography (45). It could be argued that CCK is not acting on vagal afferent fibers, but that the nodose neuronal response to BPJ diversion may be a result of an increase in muscle tone, secondary to the action of CCK on the muscle itself or the enteric cholinergic pathways. Alternatively, CCK may directly act on CCK receptors located on the pyloric sphincter. Recent evidence (13) showed that CCK (50 pmol) reduced the afferent traffic emanating from gastric tension receptors after relaxation of the stomach. Similarly, acute pylorectomy failed to block the gastric vagal afferent response to CCK (100 pmol) in rats (40), suggesting that the vagal afferent response to endogenous CCK is mediated through the action of CCK on vagal pathways. As in animals, a human study suggests that CCK at physiological concentrations induced relaxation and increased gastric wall compliance (47). Another study showed that duodenal nutrient exposure induced antral and duodenal contractions and activated antral and duodenal load-sensitive vagal afferents. The neural circuitry mediating the contractions is unclear (44). In contrast, BPJ diversion did not cause antral or duodenal contractions (T. Takahashi, Y. Li, and C. Owyang, unpublished observations).

CCK has been shown to depolarize rat nodose ganglion cells in vitro (11). In this study, after vagal branches transection, some neural discharges were still observed. Presumably this may be due to circulating CCK, which may act directly on nodose ganglion cells. It is noteworthy that potential signaling pathways from enteric neurons to vagal afferents of the rat stomach have been identified by neuroanatomic tracing studies (6). It is therefore conceivable that CCK may increase vagal afferent activities through the enteric neurons.

We conclude that under physiological conditions CCK acts on both high- and low-affinity CCK-A receptors on distinct vagal afferent fibers. The vagal CCK-receptor field includes the regions innervated by the gastric, celiac, and hepatic vagal branches. It is conceivable that activation of the vagal CCK receptors in different sites and different affinity states may mediate different digestive and metabolic functions.

This investigation was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-48419 and DK-34933 (C. Owyang) and DK-51717 (Y. Li).

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Received 25 September 1998; accepted in final form 24 April 1999.

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