Secretin activates vagal primary afferent neurons in the rat: evidence from electrophysiological and immunohistochemical studies

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Li, Ying, Xiaoyin Wu, Harry Yao, and Chung Owyang. Secretin activates vagal primary afferent neurons in the rat: evidence from electrophysiological and immunohistochemical studies. Am J Physiol Gastrointest Liver Physiol 289: G745–G752, 2005. First published May 26, 2005; doi:10.1152/ajpgi.00039.2005.—In this study, we evaluated the vagal afferent response to secretin at physiological concentrations and localized the site of secretin’s action on vagal afferent pathways in the rat. The discharge of sensory neurons supplying the gastrointestinal tract was recorded from nodose ganglia. Of 91 neurons activated by electrical vagal stimulation, 19 neurons showed an increase in firing rate in response to intestinal perfusion of 5-HT (from 1.5 ± 0.2 to 25 ± 4 impulses/20 s) but no response to intestinal distension. A close intra-arterial injection of secretin (2.5 and 5.0 pmol) elicited responses in 15 of these 19 neurons (from 1.5 ± 0.2 impulses/20 s at basal to 21 ± 4 and 43 ± 5 impulses/20 s, respectively). Subdiaphragmatic vagotomy and perivagal application of capsaicin, but not supranodose vagotomy, completely abolished the secretin-elicited vagal nodose neuronal response. In a separate study, 9 tension receptor afferents among 91 neurons responded positively to intestinal distension but failed to respond to luminal 5-HT. These nine neurons also showed no response to administration of secretin. As expected, immunohistochemical studies showed that secretin administration significantly increased the number of Fos-positive neurons in vagal nodose ganglia. In conclusion, we demonstrated for the first time that vagal sensory neurons are activated by secretin at physiological concentrations. A subpopulation of secretin-sensitive vagal afferent fibers is located in the intestinal mucosa, many of which are responsive to luminal 5-HT.

Vagal afferent; nodose ganglia; intestinal mucosa; vagotomy

THE HORMONE SECRETIN regulates pancreatic exocrine secretion of fluid and bicarbonate, gastric acid secretion, and gastric motility. The vagus nerve, particularly its afferent pathway, plays an essential role in secretin’s physiological actions (1). We and other investigators have demonstrated that secretin at physiological doses acts on vagal afferent pathways originating in the gastrointestinal mucosa to induce gastric relaxation (10) and gastric acid and pancreatic (4, 5) secretion. Furthermore, we (11) have also shown that at low rates of HCl infusion, gastric relaxation is mediated primarily by endogenous secretin, which acts through the vagal afferent pathway. Electrophysiological recording of nodose ganglia neurons has shown that under physiological conditions, CCK acts on both high- and low-affinity CCK-A receptors on distinct vagal afferent fibers (9). Similar to CCK receptors, secretin receptors are present on vagal afferent fibers, and axonal transport of secretin receptors occurs in the abdominal vagal branches (18). It is conceivable that secretin may act through similar nodose sensory neural pathways to stimulate pancreatic and gastric acid secretion and inhibit gastric motility.

The electrophysiological behavior of vagal afferent neurons in response to secretin at physiological concentrations is not known. Demonstration of a vagal sensory neuronal response to secretin will provide the functional basis to support our in vivo observation. In this study, we evaluated the vagal afferent response to physiological concentrations of secretin by performing electrophysiological studies in rats. The discharges of vagal sensory neurons supplying the gastrointestinal tract were recorded from nodose ganglia. Nodose neurons with mucosal and muscle tension receptors innervating the intestine were identified. Graded doses of secretin were administrated by close intra-arterial injections. Acute vagotomy and chemical ablation studies were performed to identify the vagal afferent fibers responsible for nodose neuronal activity in response to intestinal mucosal stimulation and administration of secretin. To exclude the influence of vagal efferent elements, secretin studies were performed in rats with chronic unilateral supranodose vagotomy. Immunocytochemical studies showed that secretin induced c-Fos expression in vagal nodose neurons, and subdiaphragmatic vagotomy and perivagal application of capsaicin prevented this expression.

MATERIALS AND METHODS

Materials

Capsaicin, atropine, and hexamethonium were purchased from Sigma Chemical (St. Louis, MO); secretin and 5-HT were purchased from Peninsula Laboratories (Belmont, CA).

Animal Preparation

Experiments were performed on adult male Sprague-Dawley rats weighing 270–350 g (Harlan; Indianapolis, IN) that had been given free access to food and water. The animals were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body wt, respectively). Supplemental doses of anesthetic agents were administered as needed to maintain a deep level of anesthesia and muscle relaxation. Under these experimental conditions, the animals exhibited no reflex response to tail pinching, and respiration was markedly depressed (shallow, irregular, <50 breaths/min). A tracheal tube permitted 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. The subdiaphragmatic vagus nerve was stimulated with a pair of Teflon-coated, pure gold wire stimulating electrodes (outside diameter, 76 μm) placed around the anterior and posterior trunks, about 2–3 cm above the gastroesoph-
ageal junction and above the accessory and celiac branches of the vagus nerve. These stimulating electrodes were loosely sutured to the esophagus to limit displacement. An overdose of anesthetic was administered to kill the animals at the end of the experiments. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

**Recording of Single Nodose Neuronal Activity**

Rats were placed in a Kopf small animal stereotaxic frame. Body temperature was maintained with a special heating pad. The right nodose ganglion was exposed by a short dorsal approach. With the use of an operating microscope, the ganglion sheath was removed and separated from the adjacent cervical sympathetic trunk and carotid artery. Protease (type XIV, 0.3 mg/ml) was applied to the ganglion for 15 min. The recording microelectrodes were pulled from glass capillaries (A-M Systems; Everett, WA) using a micropipette puller and microelectrode beveler to obtain tips ranging between 0.08 and 0.1 μm in diameter with a resistance of 40–50 mΩ. The beveled glass micropipette was filled with 1.0 M KCl and lowered into the nodose ganglion. When an activated nodose ganglion neuron was identified, the responses of that neuron to an intrarterial injection of secretin and to an intraduodenal perfusion of 5-HT solution were measured. A reference electrode was placed on an incision in the skin near the recording electrode. We recorded only gastrointestinal C-fibers, which were identified according to the following parameters measured in response to electrical stimulation of the abdominal vagus nerve: latency (60–80 ms), conduction distance between the stimulating electrode and the nodose ganglion (0.06 m), and conduction velocity (<1.0 m/s). Neuronal discharges recorded extracellularly were amplified by an AM system high-input impedance preamplifier, monitored with an oscilloscope and audio monitor, and displayed and stored on an IBM-compatible computer using Axon tape software (8, 22). The basal discharge was monitored for 2 min to confirm the stability of the basal firing frequency. The firing pattern produced by each neuron and the amplitude and waveform of each spike were monitored to ensure consistency.

**Experimental Design**

Vagal afferent fibers innervate the intestinal mucosa and muscle layer. Nodose ganglion neurons that respond to distension probably innervate the muscle layer, whereas those that respond to intestinal chemostimulation innervate the mucosa. We (22) have previously shown that duodenal distension elicits a powerful increase in nodose neuronal discharge frequency; however, these same neurons fail to respond to intraluminal perfusion of 5-HT. In the present study, to characterize the nodose neurons activated by secretin, we examined the responses of secretin-sensitive neurons to intestinal distension and luminal perfusion of 5-HT.

**Vagal nodose neuronal response to intestinal perfusion of 5-HT.** A 20-cm segment of the small intestine, including the entire duodenum and proximal jejunum, was isolated between two cannulas positioned at 4 cm (PE 60; 0.76 mm inner diameter and 1.22 mm outer diameter) and 24 cm (PE 190; 1.19 mm inner diameter and 1.7 mm outer diameter) from the pylorus. After a 15-min basal period, 1.5 ml 5-HT (10^{-3} M) was perfused into the duodenum over 1 min. Free intestinal drainage prevented an increase in intraluminal pressure. Control and test solutions were delivered separately over 1 min and washed out with isotonic saline. Recording of the nodose ganglia neurons was continued for 5 min with a 30-min resting period between experiments.

**Vagal nodose neuronal response to intestinal distension.** To characterize the nodose neurons activated by secretin, we examined the response of secretin-sensitive neurons to intestinal distension. Distension of the closed intestinal loop was achieved by closing the distal cannula and infusing 2–3 ml isotonic saline into the 20-cm isolated segment of the intestine. Intraintestinal pressure was monitored by connecting the distal catheter and a three-way stopcock to a pressure transducer. Animals with a cannula inserted into a nondistended duodenum (0 mmHg) served as controls. The saline infusion increased luminal pressure to 6–8 mmHg. Ness and Gebbatt (14) evoked visceroautomotor responses to a nociceptive stimulus at an intraluminal colonic pressure >22 mmHg. Renenh and colleagues (16) reported that in rats with an intact vagus nerve, an intestinal luminal pressure >13 mmHg evoked a decrease in the mean systemic blood pressure from 100 to 95 mmHg, suggesting a nociceptive intestinal response. Therefore, under our experimental conditions, distension induced by a luminal pressure of 6–8 mmHg is likely a nonnoxious stimulation.

Response of nodose ganglia neurons to secretin. *Intra-arterial injection of secretin.* The common hepatic artery was exposed and temporarily ligated. The gastroduodenal artery was punctured at its junction with the common hepatic artery. The catheter was inserted and threaded into the superior duodenal artery ~0.5 cm past the gastroepiploic artery (3). It was fixed in place, and the punctured wound was sealed by application of cyanoacrylate glue at the point of entry. All nodose ganglia neurons that exhibited a positive response to stimulation with luminal 5-HT or pressure distension were investigated at random with two doses of secretin (2.0 and 5.0 pmol), and changes in firing rates were recorded. Preloaded catheters prevented the need for washin. Each injection was administered in <2 s, with a 15-min interval between injections.

**Effect of bilateral subdiaphragmatic vagotomy on the vagal nodose neuronal response to secretin.** To demonstrate that secretin acts through subdiaphragmatic vagal pathways, acute bilateral subdia-

phragmatic vagotomy was performed. Before the rats were placed in a stereotaxic frame, the subdiaphragmatic vagal trunks were exposed below the stimulating electrodes. The anterior and posterior trunks of the vagal nerves were carefully dissected, and 3-0 silk sutures were passed around the nerve trunks. After nodose neuronal responses to secretin were observed, vagal transections were performed by ligating the suture and pulling the sutures out (9). Nodose neuron recording studies were repeated as previously described.

**Effect of supranodose vagotomy on the nodose neuronal response to secretin.** Vagal efferent fibers pass through the nodose ganglion in anatomically distinct ventral bundles. To eliminate the influence of vagal efferent fibers on the nodose ganglia responses evoked by secretin, unilateral supranodose vagotomy was performed in a separate group of rats. Each rat was placed in a small animal Kopf stereotaxic frame. The right nodose ganglion was exposed as previously described, and the vagal nerve was transected 3 mm above the ganglion. Recording of the right nodose ganglia neurons was performed 5 days after surgery.

**Perivaginal application of capsaicin.** To study the role of the vagal afferent pathway in the mediation of secretin’s action, a separate group of rats was treated with a perivaginal application of capsaicin. After anesthesia, the abdominal vagal trunks were exposed. A small piece of gauze soaked in 1% capsaicin solution (0.1 ml/rat) was placed on the vagal trunks for 30 min. Vehicle alone (a solution of Tween 80 and olive oil) was applied to the vagus of the control animals. Vagal afferent fiber responses as previously described were performed 7 days after surgery.

**Atropine and hexamethonium studies.** To rule out the possibility that the nodose neuronal response to secretin was a result of an increase in muscle tone, secondary to the action of secretin on muscle tone, we performed additional studies using atropine to prevent gastric and intestinal contraction. Atropine (100 mg·kg^{-1}·h^{-1}) was infused 10 min before the injection of secretin. Similar studies were performed with hexamethonium (15 mg/kg bolus plus 7.5 mg·kg^{-1}·h^{-1} continuous infusion) to examine the role of presynaptic cholinergic neurons in the mediation of secretin-stimulated nodose neuronal firing.

**Neurophysiological analysis.** Nodose neuronal activity was recorded for a 1-min period before the intestinal perfusion of 5-HT, intestinal distension, or intra-arterial administration of secretin to
and fixed for 2 h on ice. The tissue blocks were subsequently washed three times in PBS-azide and then exposed for 1.5 h to goat anti-rabbit serum conjugated to Cy3 (1:100). Sections were rinsed thoroughly in PBS, mounted on gelatin-coated slides, and coverslipped. Sections were examined using a Zeiss 400 LSM laser scanning confocal microscope (19). As an immunohistochemical control, the antisera were preabsorbed with 100 μg/ml of synthetic c-Fos protein, which almost completely abolished c-Fos immunoreactivity in all nodose ganglia studied. c-Fos immunoreactivity was not detected when the primary antiserum was omitted on sections of nodose ganglia.

quantify the resting discharge and for the 3-min period after stimulation. The means and SDs of nodose neuronal firing per 20 s during the control period were compared with 20-s maximal activity after the stimulation. A neuron was considered stimulated if the maximal activity after luminal perfusion of 5-HT, duodenal distension, or infusion of secretin increased >2SD over the mean discharge in the control period. Results were compared with those obtained after pharmacological or surgical interventions. Results are expressed as means ± SE. Statistical significance was evaluated using the appropriate Student’s paired or unpaired t-test. Multivariate ANOVA methods were used to evaluate the effect of the repeated measurement over time and treatment effect followed by Newman-Keuls tests (In Stat Biostastic 2.01, Graphpad Software). P < 0.01 was considered statistically significant.

Immunohistochemistry. Immunohistochemical studies were performed to examine c-Fos expression in vagal primary afferent neurons in response to secretin stimulation. All experiments were performed in conscious nonfasted rats between 10:00 AM and 12 PM. Rats were injected intraperitoneally with either vehicle (saline) or secretin (20 μg/kg). The rats were euthanized with an overdose of pentobarbital in 0.9% NaCl (60 mg/ml ip) 1 h after termination of the secretin injection. A transcardical perfusion through the heart or the ascending aorta was administered, first with ice-cold heparinized PBS (10 mM, pH 7.4) and sodium (60 mg/ml ip) 1 h after termination of the secretin injection. A very low level of spontaneous activity.

Fig. 1. Effects of intraluminal perfusion of serotonin (5-HT) or an intra-arterial injection of secretin on the discharge frequency of vagal nodose neurons. The nodose neurons innervating the intestinal mucosa have a very low level of spontaneous activity. A: intraintestinal infusion of 10⁻⁵ M 5-HT increased the nodose neuronal discharge frequency. The activity was maintained for the duration of the stimulus and ceased after the lumen was rinsed with isotonic saline. A close intra-arterial injection of secretin stimulated the same neuron as shown in A. Secretin administration at doses of 2.5 (B) and 5.0 pmol (C) increased the discharge frequency in a dose-dependent manner.

Table 1. Summary of responses of individual neurons to intraintestinal infusion of 5-HT, intestinal distension, and intra-arterial infusion of secretin

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Hex, hexamethonium; +, increased firings; 0, no response; −, not tested.
Fig. 2. Effects of intestinal distension or an intra-arterial injection of secretin on the discharge frequency of vagal nodose neurons. 
A: intestinal pressure distension elicited a powerful, short-latency increase in discharge frequency, which was maintained throughout the period of distension. 
B: the same neuron as shown in A failed to respond to an intra-arterial injection of secretin. 
C: administration of atropine had no effect on distension-evoked nodose neuronal firing.

Fig. 3. Effect of hexamethonium on nodose neuronal firing induced by an intra-arterial injection of secretin. 
A and B: secretin administration dose dependently increased the discharge frequency of nodose ganglion neurons. The secretin-evoked responses were characterized by a burst of action potentials with short latency (5 s), peaking within 12 s, and lasting up to 40–50 s. 
C and D: administration of hexamethonium had no effect on secretin-evoked nodose neuronal firing.
Quantitative and statistical analysis. Representative cumulative numbers of c-Fos-immunoreactive cells were obtained by counting every third section (30 μm apart, 10–11 sections/ganglion, ×40 magnification) from both left and right nodose ganglia of each animal and presented as the mean total number of c-Fos-positive neurons per section. To be counted as c-Fos positive, the cell nucleus had to be round or ovoid and stained dark brown to black compared with the surrounding cytoplasm and in contrast to nonlabeled nuclei, which are lighter than the surrounding cytoplasm. Data are expressed as means ± SE per section for all ganglia and were evaluated by the appropriate Student’s paired or unpaired t-test with Newman-Keuls tests when multiple comparisons were made. P < 0.01 was considered statistically significant.

RESULTS

Effect of Intestinal Luminal 5-HT Stimulation on Nodose Neuronal Discharge

Data were collected from the recordings of C-fibers of 91 neurons from 37 rats. All neurons previously activated by electrical stimulation of the vagus nerve were tested with intestinal perfusion of 5-HT. Mucosal afferents were either silent or displayed very low spontaneous activity (0–3 impulses/min) before the first infusion of 5-HT (Fig. 1A). The 5-HT solution (10−5 M, 1.5 ml/min) infused into the intestine evoked an increase in discharge frequency from 1.5 ± 0.2 to 25 ± 4 impulses/20 s (Fig. 1A) in 19 of 91 neurons (Table 1, neurons 1–19). Activation occurred with a short latency (mean, 3 ± 0.7 s) and reached a maximum within 30 s. After the intestinal lumen was rinsed with buffer solution, the discharge decreased gradually to basal. Neurons that showed increased basal discharge frequency (i.e., >5 impulses/20 s) were not tested further, and their data were not included. All 19 units were tested with an intra-arterial administration of secretin.

Effect of Intestinal Distension on Vagal Afferent Discharge

Intestinal volume distension evoked a response in 9 of 91 units (Table 1, neurons 20–28). Most tension receptors showed a resting discharge (6 ± 4 impulses/20 s) in the absence of any intestinal stimulation. Distension elicited a powerful short latency increase in discharge frequency (32 ± 7 impulses/20 s), which was generally maintained throughout the period of distension (Fig. 2A). These responses were not affected by the administration of atropine (Fig. 2C) or hexamethonium.

Sensitivity of Intestinal Mucosal and Muscle Tension Receptors to Secretin

Of the 19 units that responded to intraluminal perfusion of 5-HT, 15 neurons responded to a close intra-arterial injection of secretin (Table 1, neurons 1–19). Secretin at doses of 2.5 and 5.0 pmol elicited an increase in vagal afferent discharge from 1.5 ± 0.2 to 21 ± 4 and 43 ± 5 impulses/20 s, respectively. The response was dominated by a brief but intense burst of action potentials. Examples of original action potential recordings are presented in Figs. 1, B and C, and 3, A and B. These responses were not affected by the administration of atropine or hexamethonium (Fig. 3C). In a separate study, 9 of 91 neurons that did not respond to secretin were sensitive to intestinal distension (Table 1, neurons 20–28). In contrast to the vagal mucosal receptors that were activated by secretin stimulation, all of the distension-sensitive neurons failed to respond to the administration of secretin (Fig. 2B). The mean discharge frequencies of nodose ganglia neurons in response to luminal-5-HT, intestinal distension, and secretin are presented in Figs. 4 and 5.

Subdiaphragmatic Vagotomy

Data were collected from the recordings of five neurons. After acute vagotomy, all neurons previously activated by...
secretin administration were retested. Acute vagotomy abol-
ished the neuronal responses to secretin (Figs. 4 and 6C).

**Perivagal Application of Capsaicin**

Eight rats received a perivagal application of capsaicin, and
seven rats were pretreated with a vehicle solution. Experiments
were performed 7 days after treatment. In the vehicle-treated
rats, of the 39 neurons that responded to electrical vagal
stimulation, 9 were responsive to luminal 5-HT stimulation.
Five of the nine units tested increased their discharge in
response to a 5 pmol dose of secretin (basal, 1.3 ± 1 to peak,
39 ± 8 impulses/20 s). In the rats treated with perivagal
capsaicin, none of the 54 electrically stimulated neurons re-
sponded to the administration of secretin (Fig. 4).

**Supranodose Vagotomy**

Unilateral supranodose vagotomy was performed in six rats.
Recordings of the right nodose neurons were made 6 days after
surgery. Data were collected from 37 neurons (n = 7 rats) that
responded to electrical vagal stimulation. Six of thirty-seven
neurons responded to luminal 5-HT perfusion. Four of six
neurons increased their discharge in response to an injection of
secretin at a dose of 5.0 pmol (basal, 1.0 ± 1 to peak, 43 ± 6
impulses/20 s; Fig. 4).

**c-Fos-Immunoreactive Neurons in Nodose Ganglia**

Immunohistochemical studies showed that in conscious fast-
ing rats, a few c-Fos-positive neurons were visualized after an
intraperitoneal injection of saline (Fig. 7A), and the results
were not statistically different from those observed in unstimu-
lated basal conditions (data not shown). In rats with an intact
vagus nerve, the injection of secretin (5.0 pmol) increased the
number of c-Fos-positive neurons in nodose ganglia from a
basal of 3.2 ± 0.5 to 36 ± 3.0 cells/section, which represented
30% of the total number of nodose ganglia neurons. Staining
intensity varied from light to strong (Fig. 7B). Cells expressing

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**Fig. 6.** Effect of acute vagotomy on secre-
tin-evoked nodose neuronal responses. *A* and
*B*: administration of secretin dose depen-
dently stimulated nodose neuronal firings in
the rat with an intact vagus nerve. *C*: after
acute vagotomy, the same neuron as shown
in *A* and *B* failed to respond to an intra-
arterial injection of secretin.

**Fig. 7.** c-Fos immunofluorescence in vagal nodose ganglia neurons in response to an intra-arterial injection of secretin. *A*: administration of saline did not
stimulate c-Fos expression in nodose ganglia neurons. *B*: administration of secretin significantly increased c-Fos expression in nodose ganglia. *C*: vagotomy
abolished secretin-stimulated c-Fos expression in nodose neurons.
The physiological significance of these observations remains to be addressed. The electrophysiological behavior of nodose ganglia neurons in response to secretin has heretofore not been studied.

Mei (12) conducted extracellular recordings of cell bodies in nodose ganglia of cats and proved it to be a sensitive and useful technique, readily providing information about unmyelinated fibers, which are difficult to tease from the fine visceral nerve fibers. We (8, 9, 22) applied the nodose ganglia recording technique in the anesthetized rat and demonstrated the exquisite sensitivity of vagal sensory neurons to CCK and serotonin.

In this study, we evaluated the vagal afferent neuronal response to secretin in the rat and localized the sites of action of secretin on vagal afferent pathways. Single nodose neuronal discharges were recorded in anesthetized rats. The gastrointestinal receptors usually are classified into three groups: mucosal, muscle, and serosal receptors. Mucosal receptors lie in or immediately below the mucosal epithelium and detect the physical and chemical nature of luminal contents. Muscle receptors are located deep in the muscularis externae and are influenced by changes in muscle tension. In the present study, an intraintestinal perfusion of 5-HT was used as a tool to identify vagal mucosal afferent neurons (6, 7, 19, 22). Most mucosal afferent fibers identified did not exhibit spontaneous activity. Electrical vagal stimulation activated 91 neurons, and an intestinal infusion of 5-HT evoked an increased firing rate in 19 of these 91 neurons. The response had a short latency (<5 s), reached its maximum within 30 s, and returned to basal after the intestinal lumen was rinsed with isotonic saline. These 19 neurons failed to respond to intestinal distension. We then examined the sensitivity of these vagal mucosal afferents to stimulation with exogenous secretin. The major finding of the present study is the high sensitivity of some nodose ganglia neurons supplying the rat small intestinal mucosa to systemic administration of secretin. Intragastric administration of very small doses of secretin elicited dose-dependent responses in nodose ganglia neuronal activity. In contrast, none of the nodose ganglia neurons that responded to intestinal distension (i.e., muscle receptors) were sensitive to secretin. These observations suggest that secretin at physiological doses acts on intestinal vagal mucosal afferent receptors but not on muscle tension receptors. This is the first demonstration of activation of vagal afferent neurons by a systemic administration of secretin.

The responses of nodose neurons are confined to those neurons terminating within the mucosa. These electrophysiological findings provide the functional basis to support our in vivo observation that secretin at physiological doses inhibits gastric motility via vagal afferent pathways (10, 11).

SECRETIN

At the time of its discovery, secretin was assumed to act in an endocrine fashion; neuronal action was not considered (1). Supraphysiological doses of secretin and pharmacological concentrations of HCl were used in the early studies. More recently, capsaicin-sensitive vagal afferent pathways were recognized to be critical in mediating the physiological effects of secretin on exocrine pancreatic secretion (4) and gastric acid secretion (5). We (10) have shown that secretin acts on vagal afferent pathways originating from the gastroduodenal mucosa to induce gastric relaxation. At low HCl infusion rates, gastric relaxation is mediated by endogenous secretin, acting through capsaicin-sensitive vagal afferent pathways (11). An in vitro autoradiography study (18) showed that the concentration of secretin-binding sites was highest proximal to the site of the vagal ligature and declined with distance from that site. The accumulation of binding sites proximal to the ligature was four times that observed distal to the ligature, indicating that secretin receptors are not only present but are being transported toward the periphery of the vagus nerve (18). A recent study (21) demonstrated that secretin (40 or 100 μg ip) induced an increase in the number of c-Fos-positive neurons in several brain nuclei including the nucleus tractus solitarii (NTS). These effects were abolished by vagotomy. Furthermore, secretin receptor mRNA was expressed in the nodose ganglion (21). On the other hand, whole cell patch recording in medullary slice preparations has shown that NTS neurons were directly activated by secretin (20), suggesting that secretin receptors may be present in the brain stem; however, the physiological significance of these observations remains to be

![Figure 8](http://ajpgi.physiology.org/) **Fig. 8.** Effect of vagotomy on c-Fos expression of rat nodose neurons in response to secretin stimulation. *P < 0.005.
have transiently raised plasma secretin levels above the physiological range; however, the local concentration of secretin secreted by S cells in the mucosa to stimulate the vagal mucosal afferent terminal receptors is likely higher than the concentration obtained from the bloodstream after an intravenous administration. Therefore, it is likely that an intra-arterial bolus administration of secretin at doses of 2.0 and 5.0 pmol mimics local postprandial secretin concentration in the mucosa to stimulate vagal afferent neuronal discharges.

Expression of the immediate-early gene product c-Fos has been widely used as a marker of neural activity in the brain. Because Fos expression is relatively rapid and easily detected by immunohistochemical study of the cell nucleus, this method has become an invaluable tool in identifying neuronal activation (2, 17, 19) and has been used extensively in vivo studies to map postsynaptic activation of the nervous system. Fos expression in vagal primary afferent neurons has been reported to map postsynaptic activation of the nervous system. Fos formation (2, 17, 19) and has been used extensively in in vivo studies by immunohistochemical study of the cell nucleus, this method has become an invaluable tool in identifying neuronal activation. Fos expression is relatively rapid and easily detected by immunohistochemical study of the cell nucleus, this method has become an invaluable tool in identifying neuronal activation.

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In the present study, we showed that an intra-arterial injection of secretin increased c-Fos protein expression in nodose neurons of conscious rats. Subdiaphragmatic vagotomy abolished nodose neuronal c-Fos expression in response to secretin. These observations further support our electrophysiological finding that nodose ganglia are activated by the administration of low doses of secretin.

GRANTS

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