Duodenal neurons provide nicotinic fast synaptic input to sphincter of Oddi neurons in guinea pig

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MAWE, Gary M., and Audra L. Kennedy. Duodenal neurons provide nicotinic fast synaptic input to sphincter of Oddi neurons in guinea pig. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G226–G234, 1999.—We have investigated the existence of neural connections between the duodenum and the sphincter of Oddi (SO). Stimulation of duodenal myenteric fiber bundles elicited synaptic responses in SO neurons, which included nicotinic fast excitatory postsynaptic potentials (EPSPs), slow EPSPs, and α2-adrenoreceptor-mediated inhibitory postsynaptic potentials. After 48 h in organ culture, when extrinsic fibers had diminished, only the fast EPSPs persisted. Duodenal mucosal stimulation also elicited nicotinic fast EPSPs in SO neurons. There was no association between the SO neurons that received duodenal input and their chemical coding. A reciprocal projection also exists from the SO to the duodenum. In acute and cultured preparations, duodenal myenteric stimulation caused antidromic responses in 20% of SO neurons. Furthermore, 45.6 ± 10.5 neurons in SO ganglia were retrogradely labeled from dye application sites in the duodenum. It is proposed that bidirectional neural communication occurs between the duodenum and the SO and that duodenal neurons provide excitatory fast synaptic input to SO neurons through a reflex that can be activated at the duodenal mucosa.

enteric nervous system; myenteric plexus; autonomic ganglia; biliary tract

THE SPHINCTER OF ODDI (SO) is a smooth muscle sphincter located at the junction of the common bile duct and the duodenum. It plays an important role in regulating the flow of bile from the biliary tree into the intestine and in inhibiting reflux of intestinal contents into the bile or pancreatic ducts. Between meals, SO tone routes bile to the gallbladder where it is concentrated and stored. Postprandially, a coordinated contraction of the gallbladder and decreased SO resistance enable bile flow into the duodenum where it aids in the digestion of fat. Although thought to be both hormonal and neural in nature, the exact mechanisms of SO regulation are not understood.

Several lines of evidence support the concept that a neural projection exists from the duodenum to the SO. A relationship between SO activity and duodenal peristalsis has been reported, and a neural connection between the duodenum and the SO has been proposed (14, 23, 24). Furthermore, distension of the duodenum in the dog (29) and cat (25) alters SO tone.

Recently, physiological and morphological studies have been carried out to directly test for the existence of a neural projection that might underlie these physiological responses. Saccone and colleagues (17, 18) have performed both in vivo and in vitro motility experiments examining a neural connection between the duodenum and the SO. Both in vivo balloon distension and electrical field stimulation in the Australian possum were found to elicit an excitatory response in the SO. They hypothesized that this response may involve one or more intramural pathways (17). An additional in vitro investigation by the same group demonstrated that duodenal electrical field stimulation produces a frequency-dependent increase in sphincter pressure that is sensitive to hexamethonium and TTX, indicating that the response is neurally mediated and involves nicotinic neurotransmission (18).

Retrograde labeling studies in the guinea pig and Australian possum have established that there is a neural projection between the duodenum and the SO. In the Australian possum, in vivo injection of 1,1'-didodecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) into the wall of the SO also resulted in retrograde labeling of neurons in the duodenal myenteric plexus (16). We have recently demonstrated that specific subpopulations of guinea pig duodenal myenteric neurons project to the ganglionated plexus of the SO in guinea pigs (10). About 110 neurons project to the SO in a given preparation, and these neurons express choline acetyltransferase immunoreactivity but not nitric oxide synthase (NOS) immunoreactivity. Furthermore, ~20% of these projection neurons are immunoreactive for calbindin, a marker of intrinsic primary afferent neurons in the guinea pig enteric nervous system (5, 19). The duodenum-SO projection neurons are depolarized by CCK (10). Because calbindin-immunoreactive myenteric neurons send a projection to the mucosa (5) and postprandial release of CCK also occurs in the mucosa, it is possible that the duodenum-SO neural circuit could be activated by postprandial release of mucosal CCK.

Recent morphological findings confirming a neural link between the duodenum and the SO have lead to the following questions, which are addressed in the current study: 1) What types of neural signals from the duodenum are received by SO neurons? 2) Can the duodenum-SO neural circuitry be activated at the level of the duodenal mucosa? and 3) Does a reciprocal projection exist from the SO to the duodenum?

METHODS
Tissue Preparation

Adult guinea pigs (200–350 g) of either sex were anesthetized with halothane and exsanguinated. This method has been approved by the Institutional Animal Care and Use Committee of the University of Vermont (protocol 97-125). The duodenum, SO, and common bile duct were removed and...
placed in iced Krebs solution. The Krebs solution, aerated with 95% O2-5% CO2, contained (in mM) 121 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 1.2 NaH2PO4, and 8 glucose. Nifedipine (5 µM) was added to suppress muscle contractions. After removal, the tissue was placed in a Sylgard-lined dish of circulating Krebs solution, and the duodenum was opened with a longitudinal incision on the side opposite the common bile duct. The duodenum was pinned mucosal side up. For some experiments, the mucosal and submucosal layers were removed with microdissection, whereas, in other experiments, the mucosa was left intact. In all cases, the SO was also opened with a longitudinal incision and the mucosal and circular muscle layers were removed to expose the ganglionated plexus of the SO. For experiments involving organotypic culture, the duodenum was dissected to the myenteric plexus-longitudinal muscle layer and the SO was dissected to expose the ganglionated plexus.

Electrophysiological Recordings

The electrophysiological methods used in this study have been described previously (26). Briefly, the dissected whole-mount preparation consisting of SO and duodenum was placed in a low-volume (2.5 ml) recording chamber and continuously bathed (10 ml/min) in aerated (95% O2-5% CO2) Krebs solution at 33–36°C.

Individual ganglia and fiber tracts were visualized using an inverted microscope (Nikon Diaphot) equipped with Hoffman modulation contrast optics. Glass microelectrodes used for intracellular recording were backfilled with neurobiotin (0.5% in 1 M KCl), and shanks were filled with 2 M KCl and had resistances in the range of 90–120 MΩ. An Axodamp 2A amplifier with bridge circuitry for intrasomal injection of electrical currents was used to record membrane potentials. Synaptic inputs were elicited in SO neurons using monopolar extracellular electrodes made from Teflon-insulated platinum wire (25 µm diameter). Stimulation duration was 0.3–0.5 ms, and stimulation frequency was 0.5–20 Hz. Antidromic activity was identified by the rapid initiation of the spike immediately following the stimulus artifact, a short duration (<5 ms) relative to fast excitatory postsynaptic potentials (EPSPs), and an insensitivity to hexamethonium.

For one set of experiments, the duodenum was dissected to the myenteric layer and the SO was dissected to its neural layer. Although the SO border could be visualized under low-power magnification (×10) as a thickened ridge of tissue, a very fine-tipped permanent marker was used to delineate the border of the SO on the bottom of the recording chamber to ensure that the precise border could be seen under magnifications. A monopolar stimulating electrode was placed on glass microscope slides, covered with Citifluor (Citifluor, London, UK), and viewed under a Zeiss fluorescent photomicroscope equipped with an HBO 100-W mercury arc lamp. A 485-nm primary filter-520-nm secondary filter combination was used to visualize FITC and Alexa 488.

Organotypic Culture

For experiments involving organ culture, dissections were performed in a horizontal laminar flow hood with sterile solutions, instruments, and dishes. After dissection, Krebs solution was replaced with culture medium consisting of DMEM-F12 containing 10% horse serum, gentamicin (10 µg/ml), amphotericin B (12.5 µg/100 ml), nifedipine (1 µM), and antibiotic antimycotic solution (1 ml/100 ml). The preparations were placed on a slowly rocking shaker table in an incubator maintained at 37°C with a humidified atmosphere (95% O2-5% CO2). The culture medium was replaced every 24 h. After 48 h, the preparations were either fixed for immunohistochemistry or placed in a recording chamber for electrophysiological investigation.

Retrograde Labeling with Dil

Retrograde labeling studies were performed using techniques similar to those developed and previously described (2, 10). Briefly, small glass beads (200–300 µm; Sigma, St. Louis, MO) were coated with a 1 mM solution of the dialky carbocyanine probe, Dil (Molecular Probes), in 100% ethanol. With the use of fine forceps, Dil-labeled beads were placed on the duodenal myenteric plexus, within 3 mm of the SO border, and gently but firmly pressed onto the tissue. The border of the SO was delineated as described above to allow accurate visualization under fluorescence microscopy. This method of Dil application enables the introduction of a high concentration of Dil to a small, restricted site. After the desired culture period (48 h), the tissue was fixed, mounted with 50% glycerol-50% PBS, and visualized with fluorescence optics using a rhodamine filter set.

Analysis of Dil-labeled preparations. Whole-mount preparations were analyzed to establish the distribution of nerve cell bodies in the SO following Dil application to the myenteric plexus. A 565-nm primary filter-590-nm secondary filter combination was used to visualize Dil. Hardware for computerized mapping of each preparation included a motorized x-y stage attached to a Zeiss fluorescent photomicroscope equipped with an HBO 100-W mercury arc lamp light source, Luvidic video hardware, and a computer equipped with Windows 95. In each preparation, the exact location of each retrogradely
labeled neuron could be recorded with a precision of 5 µm using the computerized stage mapping system and Neuronlu- cida software (MicroBrightfield, Colchester, VT).

Histochemical and Immunohistochemical Techniques

Histochemical and immunohistochemical methods were similar to those described previously (21, 22, 26, 28). After electrophysiological studies in which the neurons were injected with neurobiotin, fixed, and labeled with a fluorescent marker, some preparations were subsequently stained for NADPH diaphorase (DA) histochemical reaction product. The tissues were rinsed in PBS and then incubated for 30 min at 37°C in β-NADPH (0.06 mM), nitro blue tetrazolium (0.12 mM), and Triton X-100 (0.3%) dissolved in 0.1 M phosphate buffer. Preparations were then rinsed in PBS and mounted on glass microscope slides.

For immunohistochemical labeling, fixed whole-mount preparations were rinsed in PBS, permeabilized in 0.5% Triton X-100 for 30 min, and incubated with primary antiserum diluted in PBS (0.1 M) containing 4% goat serum and Triton X-100 for 24 h at room temperature or 48 h at 4°C. After thorough rinsing, the preparations were exposed to species-specific secondary antibodies and mounted on glass microscope slides for fluorescent visualization.

Antibodies used in the immunohistochemistry experiments included rabbit NOS-derived from brain NOS (1:250; Santa Cruz Biotechnologies, Santa Cruz, CA), rabbit calcitonin gene-related peptide (CGRP, 1:4,000; Cambridge Labs, Wilmington, DE), monoclonal mouse tyrosine hydroxylase (TH, 1:10; Boehringer Mannheim, Indianapolis, IN), streptavidin FITC (1:400; Jackson ImmunoResearch Laboratories), streptavidin Alexa 488 (1:250; Molecular Probes), goat anti-rabbit Alexa 566 (1:500; Molecular Probes), and goat anti-mouse Alexa 488 (1:250; Molecular Probes).

Numerical Analysis

Averaged values are presented as means ± SE.

Sources of Compounds

All solutions and compounds were obtained from Sigma Chemical (St. Louis, MO) with the exception of idazoxan, which was purchased from Research Biochemicals International (Natick, MA).

RESULTS

A total of 68 neurons in 30 intact preparations of SO and duodenum were studied, under different experimental conditions, using intracellular recording techniques. The passive and active membrane characteristics of these cells were consistent with earlier findings in the guinea pig SO (6, 7, 26). The ganglionated plexus of the SO contains four different types of neurons: tonic, phasic, afterhyperpolarizing (AH), and nonspiking (NS) neurons. Tonic cells are characterized by spontaneous activity and the ability to fire action potentials throughout a depolarizing current pulse. Phasic cells, which are not spontaneously active, fire one to two action potentials only at the onset of a depolarizing current pulse. AH cells are characterized by a shoulder in the repolarizing phase of the action potential and a prolonged afterhyperpolarization that is several seconds in duration. NS neurons do not generate action potentials in response to depolarizing or hyperpolarizing current pulses, but synaptic inputs and/or antidromic responses can be detected following interganglionic fiber tract stimulation (FTS). In the current study, out of a total of 68 neurons, 20 (29.4%) neurons were tonic, 18 (26.5%) were phasic, one cell (1.5%) was AH, and 29 (42.6%) were NS neurons. There was no correlation between the cell classification and the type of response to electrical stimulation; therefore, in a given set of experiments, data collected from the various cell types were pooled.

Responses of SO Neurons to Duodenal FTS

To determine whether SO neurons receive synaptic signals from duodenal neurons, interganglionic fiber tracts within the duodenum and leading toward the SO were electrically stimulated (Fig. 1). Experiments were done in acutely dissected preparations and in preparations that had been maintained in organ culture for 48 h to eliminate extrinsic nerve fibers. Neurobiotin-filled microelectrodes were used so that impaled cells could be labeled for subsequent chemical coding.

SO neuronal responses to duodenal stimulation in acutely dissected preparations. When duodenal fiber tracts were stimulated, responses were detected in 21 of 25 (84%) neurons. Responses included fast EPSPs, slow EPSPs, inhibitory postsynaptic potentials (IPSPs), and antidromic responses (see Table 1). Of the 21 neurons that responded, duodenal FTS elicited fast EPSPs in 13 cells (62%), and 6 of these also responded with antidromic activity. Slow EPSPs were detected in one of the cells that exhibited fast synaptic and antidromic activity, as well as four additional cells that also responded with IPSPs. One neuron only received slow excitatory synaptic input, and two cells only received inhibitory synaptic input. The only AH cell tested in these experiments responded to duodenal FTS with antidromic, but not synaptic, activity.

Events identified as fast EPSPs were reversibly abolished by hexamethonium (100 µM) in all eight cells that were tested (Fig. 1A). Slow EPSPs were reversibly diminished by replacing normal Krebs solution with low-calcium (0.5 mM CaCl2, 5 mM MgCl2) Krebs solution (n = 4; Fig. 1B). The slow EPSPs had an amplitude of 7.8 ± 1.3 mV and a duration of 57.4 ± 17.7 s. When membrane hyperpolarizations thought to be IPSPs were encountered, the α2-adrenoreceptor antagonist idazoxan (1.0 µM) was added to the bath, since we have previously demonstrated that IPSPs in SO ganglia are mediated by α2-adrenoreceptors (27). In all four cells tested, stimulus-induced hyperpolarizations were reversibly blocked by idazoxan (Fig. 1C).

Antidromic activity was identified by the rapid initiation of a depolarization immediately following the stimulus artifact, a short duration (~5 ms) relative to fast EPSPs, and an insensitivity to hexamethonium (Fig. 1A). Neurobiotin-filled axons could be traced from the soma to the SO-duodenal junction in two of the cells that responded to duodenal FTS with antidromic action potentials. The finding that antidromic responses can be elicited in SO neurons in response to FTS indicates...
the existence of a neural projection from SO neurons to the duodenum.

SO neuronal responses to duodenal stimulation in extrinsically denervated preparations. To verify that extrinsic nerve fibers had degenerated in extrinsically denervated preparations, CGRP and TH immunoreactivities were evaluated in control preparations and those preparations maintained in organotypic culture for 48 h. Although TH- and CGRP-immunoreactive fibers are plentiful in SO ganglia, no TH-immunoreactive neuronal cell bodies are present (27) and CGRP-immunoreactive neurons are quite sparse (28). Fibers that are immunoreactive for TH are thought to arise from the celiac ganglia, and CGRP-immunoreactive fibers, which are also immunoreactive for substance P, are thought to be extrinsic sensory fibers. Eight whole-mount preparations were cultured for 48 h before immunohistochemical processing for TH and CGRP. Immunostaining for TH and CGRP was almost completely eliminated after 48 h in culture (Fig. 2, C and D), whereas TH- and CGRP-immunoreactive fibers were abundant in control preparations (Fig. 2, A and B).

Nine whole-mount preparations were organotypically cultured before electrophysiological investigation. Of the 26 SO neurons studied, 14 (54%) responded with fast synaptic input (Fig. 3) and three of these also exhibited stimulus-induced antidromic responses (data not shown). No neurons exhibited slow EPSPs or IPSPs.

Focal Stimulation of the Duodenal Mucosa Results in Synaptic Activation of SO Neurons

To test whether a neural circuit exists from the duodenal mucosa to the ganglia of the SO, duodenal villi were electrically stimulated, whereas SO neurons were impaled with neurobiotin-filled microelectrodes (Fig. 4). Of 20 SO neurons evaluated, 12 (60%) received fast synaptic input (Fig. 4A), 2 responded with slow EPSPs (12.5%; Fig. 4B), and 2 received inhibitory synaptic input (10%; data not shown). Table 1 summarizes these results. The latency of the fast EPSP was consistently within the range of 2–6 ms. Three of the cells exhibiting fast EPSPs were exposed to hexamethonium (100 µM), and the fast EPSP was reversibly abolished in each case (Fig. 4A). Antidromic spikes were elicited in four neurons, all of which also received fast synaptic input (Fig. 4A).

To test whether the responses to villus stimulation were the result of mucosal fiber stimulation, as opposed to spread of the current to the underlying myenteric plexus, control experiments were performed. In these experiments (n = 3 preparations), the mucosa and submucosa were lifted away from the myenteric plexus [Fig. 4, left (schematic diagram)], causing destruction of any neuronal fibers projecting between the mucosa and the underlying tissue. The submucosa-mucosa layer

Table 1. Responses of sphincter of Oddi neurons to stimulation of the duodenum

<table>
<thead>
<tr>
<th>Stimulus-Induced Response</th>
<th>Control duodenal FTS</th>
<th>48-h Culture duodenal FTS</th>
<th>Mucosal stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast EPSP</td>
<td>13/24 (54%)</td>
<td>14/26 (54%)</td>
<td>12/20 (60%)</td>
</tr>
<tr>
<td>Slow EPSP</td>
<td>6/17 (35%)</td>
<td>0/10</td>
<td>2/16 (12.5%)</td>
</tr>
<tr>
<td>IPSP</td>
<td>6/24 (25%)</td>
<td>0/26</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Antidromic</td>
<td>7/25 (28%)</td>
<td>3/24 (12.5%)</td>
<td>4/20 (20%)</td>
</tr>
</tbody>
</table>

EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; FTS, fiber tract stimulation.
was then returned to its original position and pinned in place. When the mucosa in these preparations was stimulated, none of the 20 cells tested responded to electrical stimulation at voltage amplitudes comparable to those used in the intact preparations (1–30 V). The absence of synaptic responses indicates that current spread was not responsible for the synaptic responses detected in intact preparations.

Chemical coding of SO neurons receiving synaptic input from the duodenum. SO neurons that received fast synaptic input from the duodenum were injected with neurobiotin and subsequently processed for NADPH-DA reaction product and/or NOS immunoreactivity. We have previously demonstrated that there is a one-to-one relationship between NADPH-DA reactivity and NOS immunoreactivity in SO neurons (28). Five of the 19 (26.3%) cells examined after focal stimulation of duodenal fiber bundles were identified as nitrergic neurons (Fig. 5, A and B). SO neurons that received fast synaptic input in response to mucosal stimulation were injected with neurobiotin and tested for NOS immunoreactivity (Fig. 5, C–F). One of five of these cells (20%) was immunoreactive for NOS.

Retrograde Labeling of SO Neurons From the Duodenum

Due to the detection of antidromic responses to FTS in the duodenal myenteric plexus, the possibility of a neural projection from the SO to the duodenum was further examined. When DiI-coated glass beads were applied to the duodenum within 3 mm of the SO, DiI-labeled neurons were observed in the ganglionated plexus 48 h after application of DiI to the myenteric plexus (n = 8; Fig. 6, A and B). Neurons with bright, punctate cytoplasmic labeling were determined to be DiI positive. Morphologically, the majority of labeled neurons were Dogiel type I, although occasional Dogiel type II neurons were also observed. Many DiI-labeled axons could be traced from labeled cell bodies in the SO into the myenteric plexus of the duodenum. With the use of Neurulucida software, computer-generated maps of the preparations were created (Fig. 6C). In these preparations, an average of 45.6 ± 10.5 (range 21–111) neurons in the SO were labeled with DiI.

Control experiments were performed to ensure that neuronal labeling resulted from direct retrograde axo-
nal transport of the Dil. When the SO-duodenal junction was pinched with forceps to destroy communicating axons, no Dil-labeled neurons were observed in the SO. It is likely that SO neurons are labeled by active transport of Dil along the axons rather than by passive diffusion within the plasma membrane, since retrograde labeling occurs in a matter of days with active transport and requires weeks for passive transport (9).

Furthermore, when Dil was applied to the duodenum in a previously fixed SO-duodenum preparation, no labeled neurons were observed in the SO or the duodenum after 48 h in culture medium at 37°C.

DISCUSSION

This study was conducted to test the hypothesis that a functional neural connection links the duodenum with the ganglionated plexus of the SO and that this connection could form the basis of a reflex pathway originating in the duodenal mucosa. Specifically, experiments were performed to investigate whether SO neurons receive synaptic input in response to stimulation of myenteric nerve bundles and mucosal villi of the duodenum and, if so, to identify the types of synaptic events that occur. These studies revealed that duodenal neurons projecting to the SO provide nicotinic, fast synaptic input to SO neurons. Furthermore, they revealed that a reciprocal projection from the SO to the duodenum exists, since antidromic responses were detected in SO neurons when focal stimulation in the duodenum occurred. The reciprocal SO-duodenum projection was confirmed by retrogradely labeling SO neurons from dye application sites in the duodenum.

In the current study, electrophysiological characteristics of SO neurons were identified before stimulation of duodenal inputs. Neurons were identified as tonic, phasic, AH, or NS cells, based on their responses to depolarizing and hyperpolarizing current pulses. There was no correlation between the cell classification and the type of response encountered on electrical stimulation. This lack of correlation is consistent with the recent finding that no correlation exists between electrical properties and neurochemical phenotypes of non-AH SO neurons (8). It was proposed that electrophysiological phenotypes may be a reflection of the level of excitability of a neuron and that tonic and phasic phenotypes may not necessarily represent two different cell types but rather a single electrophysiological phenotype in a different state of excitability.

In the present study, stimulation of individual duodenal myenteric fiber bundles passing toward the SO elicited fast synaptic responses in SO neurons. These synaptic responses were completely blocked by hexamethonium, indicating that they involved the release of ACh onto nicotinic receptors. These data are consistent with previous findings that the duodenum-SO projection neurons are cholinergic (10) and that motility responses in the Australian possum are attenuated by hexamethonium (18). This synaptic connection is likely to involve neurons in the duodenum projecting to the SO, rather than fibers of passage, since the fast EPSPs persisted after culturing the whole mount for 48 h before examination.

In addition to the fast synaptic inputs to SO neurons, IPSPs and slow EPSPs were activated in some cells when duodenal myenteric fiber bundles were stimulated. However, it is likely that these synaptic inputs do not arise from the duodenum. Previously, it has been demonstrated that IPSPs in SO ganglia involve the release of norepinephrine from sympathetic postganglionic fibers; they are reversibly blocked by the α2-
antagonist idazoxan, and they are augmented in the presence of the catecholamine reuptake blocker desipramine (27). The IPSPs that were detected in the current study, in response to duodenal FTS, likely involve activation of the same set of nerve fibers, since they were reversibly blocked by idazoxan, and this type of synaptic event was absent in organ culture preparations.

Slow EPSPs were also detected in SO ganglia in response to stimulation of myenteric fiber bundles. The mediators of slow EPSPs in SO ganglia have not been identified, but they may involve the release of tachykinins, since substance P causes a prolonged depolarization of SO neurons (15). Moreover, slow EPSPs were not detected in response to duodenal FTS in preparations studied after 48 h in culture, when sensory fibers were extremely sparse.

Results from these studies indicate that IPSPs and slow EPSPs, activated in SO ganglia by duodenal stimulation, are likely to be initiated by extrinsic nerve fibers that travel through the plexuses of the duodenum and the SO. Recently, Hillsley and Mawe (7) demonstrated that SO ganglia have specific receptors for 5-hydroxytryptamine (5-HT) and that exogenous application of 5-HT results in prolonged depolarizations in SO neurons. However, it is unlikely that 5-HT mediated the slow EPSPs in this study, since 5-HT immunostaining persists when SO-duodenum preparations are maintained in culture for up to 6 days.

It is possible that the duodenum-SO synaptic connection could be activated by stimuli originating at the duodenal mucosa. A proportion of duodenum-SO projection neurons are calbindin immunoreactive (10), and these neurons send projections to the intestinal mucosa.

Fig. 5. Neurobiotin-filled neurons that received fast synaptic input from the duodenum were examined for nitric oxide synthase (NOS) immunoreactivity/NADPH-DA product. A and B: simultaneous demonstration of FITC in neurobiotin-filled cells (arrows) that responded to duodenal FTS and NADPH-DA (dark cells). Neither of these neurobiotin-filled cells contained NADPH-DA reaction product. C-F: neurobiotin-filled SO neurons that responded to stimulation of the duodenal mucosa. C and E: neurobiotin-filled neurons visualized with FITC. D and F: matching fields demonstrating immunoreactivity for NOS, which was visualized with Cy3. Arrows indicate the positions of the neurobiotin-labeled neurons in C and E. Note that the neurobiotin-injected cell in D was positive for NOS, whereas the neurobiotin-labeled neuron in E was negative for NOS. A: phasic cell; RMP = −40 mV; fast EPSP and antidromic action potentials detected. B: phasic cell; RMP = −51 mV; fast EPSP detected. C and D: tonic cell; RMP = −58 mV; mucosal stimulation elicited fast EPSPs and IPSPs. E and F: phasic cell; RMP = −50 mV; mucosal stimulation elicited fast EPSPs. Scale bars = 50 µm.
(3, 5, 19). The calbindin-immunoreactive neurons of the myenteric plexus are thought to serve as primary sensory neurons in the intrinsic reflex circuitry of the gut (1, 11, 12). The calbindin-immunoreactive neurons that are retrogradely labeled from the SO are likely to terminate in SO ganglia, since these ganglia are rich in calbindin-immunoreactive fibers, but calbindin-immunoreactive neurons are rare (26). Furthermore, varicos calbindin-immunoreactive nerve fibers in SO ganglia degenerate when the isolated SO is maintained in culture but persist when maintained in culture with the duodenum intact (10).

In the current study, when recordings were made from SO neurons while duodenal mucosal villi were stimulated, hexamethonium-sensitive fast EPSPs were detected in SO neurons. Synaptic responses were not observed when neural connections between the mucosa and the myenteric plexus were disrupted, indicating that spread of the electrical stimulus to the myenteric plexus was not responsible for the synaptic response. Although morphological findings, described above, indicate that a monosynaptic projection probably exists between the duodenal mucosa and the ganiglia of the SO, it is not clear whether the fast EPSPs evoked by mucosal stimulation were monosynaptic. It is possible that these events involved multisynaptic connections in the myenteric plexus and/or projections from submucosal neurons to myenteric neurons that project to the SO. It is not possible to conclusively test this experimentally, but the finding that the latencies of the EPSPs were rather brief (2–6 ms), with little variability, indicates that a monosynaptic connection was involved.

A small number of SO neurons exhibited IPSPs on duodenal mucosal stimulation; this response was probably due to activation of sympathetic nerve fibers that pass through SO ganglia and to the duodenal mucosa. Sympathetic nerve fibers pass within intestinal villi in the guinea pig small intestine (13).

When possible, SO neurons receiving synaptic input from the duodenum were neurochemically identified by testing for NOS immunoreactivity or NADPH-DA reaction product. Previous studies have determined that there are two populations of SO neurons, based on their chemical coding patterns; ~25% of the neurons are nitroergic and the remainder are cholinergic (20). These proportions closely match the proportions of neurons receiving fast synaptic input from the duodenum in the current study, indicating that there is a lack of correlation between the chemical coding of SO neurons and those receiving synaptic input from the duodenum.

Antidromic responses were elicited in SO neurons by duodenal stimulation, indicating that there is a reciprocal connection arising in the SO and projecting to the duodenum. In addition, SO neurons were retrogradely labeled when DiI was placed on the duodenum. The number of SO-duodenum projection neurons identified in this study (~45/preparation) is likely to be an underrepresentation of the SO neurons making this projection. In some of these experiments, DiI was applied to only a small region of the duodenal myenteric plexus.
Thus only SO neurons projecting to that location would be labeled. Future experiments should involve examination of the type(s) of SO neurons that projects to the duodenum.

In summary, these data demonstrate that duodenal neurons provide fast excitatory synaptic input to SO neurons. This pathway can be activated by stimulation of myenteric fiber bundles as well as duodenal villi. This latter finding supports the concept that a neural circuit, intrinsic to the gut, could convey signals from the duodenal mucosa to SO neurons. Furthermore, reciprocal connections from the SO ganglia to the duodenum were also discovered in these studies. The neural connections between the duodenum and the SO are likely to have roles in intrinsic reflex circuits of the gut enabling a coordination of SO tone and duodenal motor activity.

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