5-HT is present in nerves of guinea pig sphincter of Oddi and depolarizes sphincter of Oddi neurons

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Hillsley, Kirk, and Gary M. Mawe. 5-HT is present in nerves of guinea pig sphincter of Oddi and depolarizes sphincter of Oddi neurons. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1018–G1027, 1998.—This study involved immunohistochemistry and intracellular electrophysiology to investigate serotonergic neurotransmission in the sphincter of Oddi (SO). 5-Hydroxytryptamine (HT)-positive neurons (14 cells/preparation) and nerve fibers were observed in the ganglionated plexus. Serotonergic nerve fibers, which persisted under 2- to 6-day organ culture, were densely distributed, with varicose endings encircling some SO neurons. When 5-HT was applied to SO neurons, it elicited three different responses: 1) a fast depolarization to 5-HT in 31 of 62 cells was mimicked by 2-methyl-5-HT and blocked by LY-278584 (1 µM); 2) a prolonged depolarization to 5-HT in 21 of 62 cells evoked an increase in input resistance and was attenuated by the 5-HT$_1$P antagonist renzapride (1 µM) but not by the 5-HT$_4$ antagonist SDZ-205557 (0.1–10 µM); and 3) an indirect depolarization blocked by TTX or atrazine was observed in 32 of 62 cells. 5-HT superfusion elicited a dose-dependent monophasic depolarization (EC$_{50}$ = 2 µM, n = 14). In conclusion, 5-HT is present in nerves of the SO and elicits both 5-HT$_3$ and 5-HT$_1$P receptor-mediated depolarizations, supporting the concept that 5-HT plays a role in SO regulation.

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

THE SPHINCTER OF ODDI (SO), which is a valve that regulates the flow of bile and pancreatic secretions into the duodenal lumen, is innervated by a ganglionated plexus that lies within its wall (19). This neural plexus of the SO appears to be contiguous with the duodenal myenteric plexus, but observations that SO neurons have distinct electrophysiological and neurochemical characteristics indicate that they are distinct neural entities. SO neurons exhibit either tonic or phasic firing characteristics (27) and are made up of two principal populations of neurons according to their neurochemical characteristics (26, 29): those that are immunoreactive for excitatory neurotransmitters such as acetylcholine, substance P, and enkephalin, and those immunoreactive for inhibitory agents such as nitric oxide and vasoactive intestinal peptide. The neurons of the SO can be both intrinsically and extrinsically regulated; nerves can be externally modulated by inputs from the parasympathetic (9) and sympathetic nerves (28), by hormones such as cholecystokinin (2, 22), and possibly by neural projections from the duodenum (18, 23).

One potential neural regulator, which has been demonstrated to influence the motility of this SO function, is serotonin [5-hydroxytryptamine (5-HT)]. In organ bath preparations, 5-HT causes a concentration-dependent contraction of the guinea pig SO (7, 17), and it is likely that this is a neurally mediated event, because the contraction is significantly attenuated by atropine (1). Furthermore, Behar and Biancani (3) have demonstrated that, in the cat, intravenous infusion of 5-HT in vivo results in two different SO responses (3): one is a contraction that is partially mediated by the neural release of acetylcholine from the neural plexus of the SO, and the other is a relaxation that is mediated by the stimulation of a nonadrenergic, noncholinergic pathway. The Behar study on the cat SO provided evidence that serotonergic neurons may play a physiological role in the regulation of basal SO motor activity, because 5-HT blockade or depletion reduced the basal tonic pressures and the amplitude of phasic contractions.

Taken together, these studies suggest that 5-HT can alter SO function through actions within the local ganglionated plexus. To directly examine this, the current study was undertaken to determine whether the 5-HT is present in the nerves of the ganglionated plexus of the SO and whether SO neurons respond to the application of 5-HT and associated compounds. Evidence presented indicates that, in the guinea pig SO, 5-HT-immunoreactive nerve fibers and cells are present and that 5-HT has direct excitatory actions on SO neurons, supporting the concept that 5-HT could act as a neurotransmitter in SO ganglia.

METHODS

Tissue Preparation

Guinea pigs (200-350 g) were deeply anesthetized with halothane and then exsanguinated. The region of the SO that included the terminal portion of the common bile duct, the choledochoduodenal junction, and the adjacent duodenum was quickly removed and washed with Krebs solution. The duodenum was then cut open with a longitudinal incision on the side opposite the bile duct and pinned flat under recirculating iced Krebs solution in a dish lined with Sylgard elastomer. The mucosal layer of the duodenum was gently removed with forceps under microscopic observation. The lumen of the SO region was exposed by making a longitudinal incision from the papilla through the ampulla and along the bile duct. The SO region was gently stretched and pinned flat, and the surrounding duodenal tissues were removed. The mucosal and circular muscles of the SO region were carefully removed, exposing the ganglionated plexus. The preparation was then transferred to a 3.5-ml tissue chamber for electrophysiological recording. All recordings were made from the ganglia in the ampulla and choledochal regions of the SO.
Electrophysiological Recording

The methods for intracellular electrophysiological recording were similar to those described previously for SO neurons (27, 28). The preparation was continuously perfused (10 ml/min) with Krebs solution that contained (in mM) 121 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 8 glucose, and 0.001 nifedipine. The bathing solution was aerated with 95% O2-5% CO2, and the temperature was maintained at 36-37°C. The ganglia were visualized at a magnification of ×200 using an inverted microscope (Nikon Diaphot) equipped with Hoffman Modulation Contrast optics.

Glass microelectrodes used for intracellular recording were filled with 2.0 M potassium citrate or KCl and had resistances in the range of 60-90 MΩ. An Axoclamp 2A amplifier, equipped with bridge circuitry for intracellular injection of positive and negative current pulses, was used to record membrane potentials. Changes in input resistance were assessed by measuring electrotonic potential in response to a hyperpolarizing current pulse (0.1 nA, 0.6 Hz, 200 ms) before and during the 5-HT-induced depolarization.

Compounds were applied by either addition to the circulating Krebs solution or pressure microejection. 5-HT was pressure microprojected from glass micropipettes (1 mM in Krebs solution; 15- to 20-μm tip diameter) by pulses of nitrogen gas (20 psi, 10–500 ms in duration). Just before each pressure microejection, the tip of the micropipette was lowered to within 50 and 100 μm of the impaled neuron. A second spitzt microelectrode was aligned at a different angle but proximal to the same impaled neuron for experiments assessing the actions of 2-methyl-5-HT. When the actions of antagonists were evaluated, these compounds were added to the circulating Krebs solution at least 5 min before 5-HT microejection was tested. When progressively increasing concentrations of 5-HT were added to the Krebs solution, they were added after 10-min intermittent washes. 5-HT receptor ligands were obtained from Research Biochemicals International (Natick, MA). All other compounds were purchased from Sigma Chemical (St. Louis, MO).

5,7-Dihydroxytryptamine Pretreatment

In preliminary experiments, before fixation and subsequent immunohistochemical processing, SO preparations were incubated at 37°C with 6-hydroxydopamine (0.5 mM) for 2 h and 5,7-dihydroxytryptamine (5,7-DHT; 0.1 mM) for 30 min. However, it was found that, if neuronal staining was amplified using biotinylated secondary antibodies with a streptavidin tertiary antibody (as detailed in Immunohistochemistry), good staining was observed for 5-HT without the necessity for 5,7-DHT pretreatment. Thus, in the majority of experiments and in Figs. 1–8, the latter protocol of immunohistochemistry without 5,7-DHT pretreatment was employed.

Immunohistochemistry

SO whole mount preparations were stretched and pinned in a dissection dish and then fixed overnight at 4°C in 0.1 M sodium phosphate buffer containing 2% paraformaldehyde and 0.2% picric acid. 5-HT immunofluorescence was observed regardless of whether the SO mucosa was removed before or after fixation. The preparations were further processed by rinsing in several changes of 0.1 M PBS containing 0.5% Triton X-100. The SO preparations were incubated with primary antisera [rat anti-5-HT, 1:200 (Seralab), mouse anti-microtubule-associated protein 2 (MAP2), 1:500 (Boehringer Mannheim, Indianapolis, IN)] diluted in PBS containing 4% normal horse serum and Triton X-100 for 18-24 h at room temperature. After thorough rinsing, the preparations were exposed to species-specific secondary antibodies [indocarbocyanine (Cy3)-biotin-conjugated goat anti-rat, 1:500 (Jackson ImmunoResearch Labs, West Grove, PA); FITC-conjugated donkey anti-mouse, 1:400] and tertiary antibodies [Cy3-conjugated streptavidin, 1:400 (Jackson ImmunoResearch Labs)] when appropriate. The tissue was then rinsed, dehydrated, and coverslipped with Citifluor (Canterbury, UK). The slides were examined with a Nikon fluorescence photomicroscope equipped with an HBO 100-W ultraviolet light source. A 565-nm primary filter/590-nm secondary filter combination was used to visualize Cy3. A 485-nm primary filter/520-nm secondary filter combination was used to visualize FITC.

Maintenance of SO Preparations in Organ Culture

SO preparations were cultured for up to 6 days, either in isolation or with the surrounding duodenum intact. The mucosa and submucosa were dissected from the SO in Krebs solution under cold, sterile conditions, leaving the circular muscle intact. The Krebs solution was replaced with sterile culture medium that contained 89.9% DMEM and Ham’s nutrient mixture F-12 (DMEM/F-12), 10% horse serum, 1% antibiotic antimycotic solution, 1% gentamicin solution, 0.5% amphotericin B solution, and 0.02% nifedipine (1 mM). All solutions and compounds were obtained from Sigma Chemical (St. Louis, MO). The SO preparation was placed on a shaker table (The Waver, VWR Scientific Products, Bridgeport, NJ) that rocked at 40 cycles/min inside an incubator set to 37°C. The culture medium was replaced every 24 h. After the desired culturing period (up to 6 days), SO preparations were fixed and processed as described in Immunohistochemistry.

Statistical Analysis

Averaged values are presented as means ± SE. Student’s t-test was used to test for significance of differences between control and paired experimental data sets. Differences were considered significant where P was <0.05.

RESULTS

Data were obtained from a total of 90 SO preparations in the course of this study. Of these, 35 were utilized for immunohistochemistry and 55 for electrophysiology.

Immunohistochemical Studies

Extensive and intense neural immunostaining for 5-HT was observed in all preparations (see Fig. 1). Immunoreactive nerve fibers were densely distributed throughout the ganglionated plexus (Fig. 1A), with foci of serotonergic fibers coursing along the edges of ganglia (Fig. 1B) and occasionally through the middle of ganglia. 5-HT-immunoreactive fibers had varicose endings within SO ganglia (Fig. 1, A and C), but the degree of innervation varied within and between ganglia. The varicose endings often appeared to delineate individual neuronal cell bodies (Fig. 1C). This was confirmed by co-staining preparations with the neuronal marker MAP2, as shown in Fig. 2. In addition, varicose nerve fibers were observed in the SO circular muscle. Serotonergic neuronal cell bodies were also observed (Figs. 1D and 3). The mean number of 5-HT-
immunoreactive neurons was 14.3 ± 2.8 per SO preparation (n = 18), which measured 27.1 ± 1.4 mm² in size (0.53 cells/mm², n = 18).

To test whether the rich network of 5-HT-immunoreactive nerve fibers in the SO ganglionated plexus were intrinsic and/or from an extrinsic source, the pattern of 5-HT immunoreactivity was evaluated in 15 SO preparations that were maintained in organ culture for up to 6 days, either in isolation or with the adjacent duodenum intact (Fig. 3). In all cultured preparations, the large fiber bundles were almost entirely absent, indicating that the axons within these neuronal fascicles were from an extrinsic source. Aside from this difference, the general pattern of immunoreactivity as described above persisted in cultured SO preparations, regardless of the integrity of the duodenum or the duration of culture, indicating that the serotonergic cells and most fibers were intrinsic to the SO. However, there did appear to be a gradual reduction in the varicose serotonergic innervation as the duration of culture increased from 2 to 6 days.

Electrophysiological Studies

In the course of the electrophysiological studies, a total of 85 neurons from 55 SO preparations were impaled with intracellular microelectrodes; 5-HT was picospritzed onto 68 neurons, and 5-HT was superfused over 17 neurons. The passive and active membrane characteristics of these cells were consistent with those reported previously for guinea pig SO (16, 28). Tonic cells were readily excitable and often exhibited spontaneous activity, phasic cells were relatively unexcitable, and AH cells had a 5- to 6-s afterhyperpolarization following an action potential. Tonic and phasic cells comprise the vast majority of neurons in the SO (~90%). In the current study, the actions of 5-HT were tested on 43 tonic cells (50.6%), 35 phasic cells (41.2%),
and 7 AH cells (8.2%). There was no correlation between the cell classification and the type of response to 5-HT application, and therefore all of the data presented below were pooled.

Actions of 5-HT Applied by Pressure Microejection

SO neurons responded to 5-HT in 91.2% of the cells tested (62 of 68) and evoked three distinct responses that could occur independently or in combination in any particular cell (Fig. 4). The incidence of different combinations of 5-HT responses is shown in Table 1.

Rapid depolarization. In 31 of 62 neurons tested, application of 5-HT by pressure microejection resulted in a rapid depolarization that was typically associated with the firing of 1–2 action potentials in tonic cells (Figs. 4 and 5). The rapid depolarization had a mean duration of 1.5 ± 0.2 s (range = 0.6–3.6 s) and a peak amplitude of 9.5 ± 1.2 mV (range = 2–19 mV, n = 16) and was associated with a 75.3 ± 2.6% decrease in input resistance (n = 7; Fig. 5A). The magnitude of this type of response decreased when 5-HT was repeatedly applied at intervals of <90 s apart (n = 2). However, if the interval between successive 5-HT applications was >5 min, desensitization of the rapid depolarization was not detected.

In 15 cells, a spike was elicited without any discernible depolarization. Although all three electrophysiological cell types exhibited the rapid depolarization to 5-HT, every AH cell tested (6 of 6 neurons) responded to 5-HT in this manner.

Bath application of either 1 µM atropine (n = 5) or 100 µM hexamethonium (n = 5) failed to produce any measurable changes in the rapid depolarization elicited by 5-HT. Furthermore, no changes in the amplitude or duration of the rapid depolarization were detected in the presence of TTX (0.5 µM; n = 6), but there was an abolition of action potentials superimposed on the depolarization.

The rapid depolarization induced by 5-HT was mimicked by pressure microejection of the 5-HT3 receptor agonist 2-methyl-5-HT (1 mM) in 3 of 3 cells. Figure 5B demonstrates that, in a given cell, the duration and magnitude of the rapid depolarizations induced by 5-HT and 2-methyl-5-HT were comparable. Microejection of 5-HT in the presence of a selective 5-HT3 receptor antagonist, LY-278584 (1 µM), failed to elicit a
detectable depolarization in 12 of 12 cells (Fig. 5C). The 5-HT₂/5-HT₁C receptor antagonist ketanserin (1 µM) had no detectable effect on the rapid depolarization in 6 of 6 cells.

Prolonged depolarization. Application of 5-HT by pressure microejection resulted in a prolonged depolarization of 21 of 62 neurons (33.9%). The mean duration of the depolarization was 151 ± 19.9 s (range = 66–314 s), and the mean peak amplitude of the depolarization was 8.3 ± 1.1 mV (range = 4–19 mV). The prolonged depolarization in response to 5-HT was associated with the firing of action potentials in 10 of 21 cells (for example, see Fig. 6B). The input resistance was increased (by ~20%) during the prolonged depolarization.

The 5-HT-induced prolonged depolarization was not altered by prior superfusion with the 5-HT₃ receptor antagonist LY-278584 or by TTX superfusion (n = 3). In addition, the 5-HT₂ receptor antagonist SDZ-205557 (0.1–10 µM) had no significant effect on the prolonged depolarization to 5-HT (control = 10.7 ± 2.1 mV, SDZ-205557 = 10 ± 1.1 mV, n = 3) as shown in Fig. 5A. However, the 5-HT₁P receptor antagonist renzapride (BRL-24924; 1 µM) caused a 64% reduction in the prolonged depolarization (control = 6.2 ± 1 mV, BRL-24924 = 2.3 ± 0.8 mV, n = 6, P < 0.01), as shown in Fig. 6B.

Indirect depolarization. Application of 5-HT by pressure microejection resulted in a third type of depolarization of 32 of 62 neurons. This response had a duration of 23 ± 2 s (range = 7.5–43.2 s) and a peak amplitude of 7.3 ± 0.9 mV (range = 3–13.4 mV). This type of depolarization in response to 5-HT was associated with the firing of action potentials in only 3 of the 32 cells that responded in this manner.

Unlike the rapid and prolonged depolarizations described above, bath application of 1 µM atropine (5 of 5 cells; Fig. 6A) or 0.5 µM TTX (4 of 4 cells, Fig. 7B) totally abolished this type of 5-HT-induced depolarization. Therefore, this type of response is an indirect depolarization to 5-HT. The change in input resistance of the cell during the 5-HT-induced rapid depolarization was not consistently changed during the indirect depolarization.

Ketanserin (1 µM) had no effect on the indirect depolarization in 5 of 5 cells. The indirect depolarization induced by 5-HT was mimicked by microejection of 2-methyl-5-HT (1 mM) in 5 of 5 cells. Microejection of 5-HT in the presence of LY-278584 (1 µM) failed to elicit a response of any kind in 9 of 9 cells (Fig. 7D); this effect was readily reversible.

Table 1. Incidence of different 5-HT responses in sphincter of Oddi neurons

<table>
<thead>
<tr>
<th>5-HT Response</th>
<th>No. of Cells</th>
<th>Percentage of Cells</th>
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<tbody>
<tr>
<td>Rapid</td>
<td>10</td>
<td>16.1</td>
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<tr>
<td>Rapid/prolonged</td>
<td>2</td>
<td>3.2</td>
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<tr>
<td>Rapid/indirect</td>
<td>18</td>
<td>29.0</td>
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<tr>
<td>Rapid/indirect/prolonged</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Prolonged</td>
<td>18</td>
<td>29.0</td>
</tr>
<tr>
<td>Prolonged/indirect</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Indirect</td>
<td>13</td>
<td>21.0</td>
</tr>
</tbody>
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5-HT, 5-hydroxytryptamine.

Superfusion of 5-HT caused a concentration-dependent monophasic depolarization of SO neurons (Fig. 8). A response to 5-HT was elicited in 14 of 17 cells tested. The maximal depolarization of SO neurons to 5-HT (25 µM) was 9.5 ± 1.3 mV, which was not significantly different from the amplitude of the prolonged depolar-
Fig. 5. Characterization of 5-HT-induced rapid depolarization. A: trace showing decrease in input resistance during fast response to 5-HT, consistent with opening of a cation channel. B: fast depolarizing response to 5-HT was mimicked by microejection of 5-HT$_3$ receptor agonist 2-methyl-5-HT (2M-5-HT). Magnitudes of 5-HT- and 2M-5-HT-induced depolarizations were comparable on SO neurons. C: fast response to 5-HT was reversibly abolished by 5-HT$_3$ receptor antagonist LY-278584 (1 µM).

Fig. 6. Evaluation of prolonged depolarization to 5-HT. A: prolonged depolarizing response to 5-HT was not significantly affected by prior superfusion of 5-HT$_4$ receptor antagonist SDZ-205557 at either 0.1 or 10 µM. B: prolonged response to 5-HT was significantly attenuated by prior superfusion of 5-HT$_3$ receptor antagonist renzapride (1 µM) in this tonic cell.
DISCUSSION

This study was conducted to determine whether 5-HT is present in nerves of the SO and to directly test whether SO neurons respond to application of 5-HT. The results reported here provide evidence that 5-HT-immunoreactive nerve fibers are abundant in the ganglionated plexus of the SO and that three different types of depolarizing responses can be elicited by application of 5-HT to SO neurons. 5-HT has been extensively studied throughout the gastrointestinal tract and mediates a variety of actions such as slow excitatory postsynaptic potentials (EPSP) in enteric ganglia, peristalsis, vomiting, and secretion (15). Motor responses to 5-HT in the SO have been previously demonstrated (1, 3, 7, 17), but this is the first study to directly demonstrate the presence of serotonergic neurons and specific 5-HT receptors in SO ganglia and the first to demonstrate the serotonergic innervation of a gastrointestinal sphincter.

The pattern of 5-HT-positive nerve fibers observed in the guinea pig SO was similar to that previously described in the myenteric plexus of the guinea pig small intestine (8, 13) and was consistent with the concept that there is a specific serotonergic innervation of a subpopulation of SO neurons. Varicose terminals of 5-HT-immunoreactive fibers were not uniformly distributed either within each individual ganglion or between ganglia in the same SO preparation. The degree of innervation of a particular ganglion was not dependent on its location in the SO such that ganglia in the proximal sphincter were no more or less heavily innervated than ganglia in the distal SO.

A very dense network of 5-HT-immunoreactive fibers was observed in the ganglionated plexus of the SO relative to the small number of 5-HT-immunoreactive cells. Only ~14 cells per SO preparation were detected, whereas fibers were abundant throughout the ganglia and interganglionic fiber bundles. Wells et al. (29)

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**Fig. 7.** Characterization of indirect depolarization. A: response to 5-HT was totally abolished by atropine. B: 5-HT response was shown to be indirect because TTX completely abolished 5-HT-induced depolarization. C: slow, indirect depolarization was also abolished by 5-HT3 receptor antagonist LY-278584 (1 µM). D: there were no consistent changes elicited in input resistance during indirect depolarization.

**Fig. 8.** Dose-dependent depolarization of SO neurons. A: in 14 of 17 cells, a dose-dependent depolarization was elicited by 5-HT superfusion, with an EC50 of 2 µM. 5-HT concentration. B: example of dose-dependent 5-HT superfusion in a tonic cell. Depolarizing response to 5-HT was maintained for duration of superfusion.
reported that there are 1,855 ± 157 neurons per SO preparation, indicating that the number of 5-HT-immunoreactive cells represents <1% of cells within SO preparations. In the small intestine, ~2% of neurons are serotonergic (5). The low ratio of cells to fibers would suggest that many of these serotonergic fibers are from a source that is extrinsic to the SO. In the guinea pig small intestine, the majority of 5-HT-immunoreactive nerves are intrinsic to the gut tube, because serotonergic fibers and cells bodies survived in organotypic culture for extended periods of time (11). In the ganglionated plexus of the SO, immunoreactivity for 5-HT persisted in preparations that were maintained in organ culture for periods of up to 6 days. This is sufficient time for extrinsic innervation to degenerate, because calcitonin gene-related peptide immunoreactivity in the SO is entirely absent after three days in the same organ culture conditions (24) (A. L. Kennedy and G. M. Mawe, unpublished observations). Serotonergic cells persisted in cultured preparations, and varicose fibers were apparent, indicating that there is an extensive intrinsic serotonergic innervation providing a readily available source of 5-HT within the SO. However, the large bundles of 5-HT-positive nerve fibers were completely absent in cultured preparations, indicating that these nerve fascicles were extrinsic to the SO. The absence of these large nerve fascicles may account for any small decreases in the intrinsic innervation observed. Because the integrity of the duodenal myenteric plexus, which is contiguous with the ganglionated plexus of the SO, made no apparent difference to the pattern of cultured 5-HT immunoreactivity, it is unlikely that these nerve bundles originated in the proximal duodenum.

Within a given ganglion, only a subset of the cells appeared to be delineated by the punctate 5-HT-immunoreactive fibers, and these cells were not preferentially found at the edge or in the middle of ganglia. This apparent delineation of certain SO neurons was confirmed when double-label immunoreactivity was performed with the neuronal marker MAP2. When 5-HT and MAP2 immunoreactivities were simultaneously viewed with dual fluorescence, it was clear that the rings of varicose serotonergic fibers coincided with the outer edges of neuronal cell bodies, and thus the 5-HT-immunoreactive fibers were in close association with some specific SO neurons. This physical proximity would allow for the chemical neurotransmission of 5-HT from the axonal varicosities directly onto specific cell bodies within the SO. Thus, because there is a source of 5-HT in close proximity to a population of SO cells, and because its release from these varicosities would result in the activation of the adjacent cell body, this observation supports the theory that the effects of exogenously applied 5-HT on SO neurons observed in this study could represent the actions of neurally released 5-HT intrinsic to SO ganglia.

As might be expected from the many subtypes of 5-HT receptors that have been found to mediate the actions of 5-HT in the gastrointestinal tract (10, 12), more than one type of response to 5-HT was observed in SO neurons. Most SO neurons that directly responded to 5-HT exhibited a fast depolarization to pressure microejection of 5-HT. This was a direct action of 5-HT on SO neurons, because it could not be blocked by atropine, hexamethonium, or TTX. The rapid depolarization to 5-HT has been described in both submucosal and myenteric neurons and is caused by the stimulation of a ligand-gated cation channel by 5-HT3 receptor agonists (6, 10, 21, 25). The fast response in SO neurons was also found to be a 5-HT3 receptor-mediated phenomenon, because there was a decrease in input resistance and the response was mimicked by the 5-HT3 receptor agonist 2-methyl-5-HT and blocked by the 5-HT3 receptor antagonist LY-278584.

In the intestinal myenteric plexus, the activation of 5-HT3 receptors has been proposed to induce smooth muscle contraction by releasing acetylcholine from intrinsic cholinergic motoneurons (4). In manometric recordings from the cat SO, 5-HT was found to result in a transient contraction that was primarily caused by the stimulation of postganglionic cholinergic neurons and, to a lesser extent, a direct action on the muscle (3). However, the 5-HT receptor subtypes involved were not elucidated. Organ bath studies in the guinea pig similarly reported only contractile events in response to 5-HT (7, 17). In addition, a small twitch contraction was often observed in this study in response to 5-HT microejection. Because the majority of neurons are cholinergic in the guinea pig SO (26), it is likely that at least some of the neurons in this study, in which 5-HT3 receptor rapid depolarizations were recorded, were from cholinergic motoneurons. Therefore, it is proposed that 5-HT3 receptors on cholinergic motoneurons are putative mediators of SO contractile activity.

Evidence presented in this study suggests that 5-HT3 receptors may also be located on SO interneurons. A response to locally applied 5-HT recorded in SO neurons was initiated by the stimulation of 5-HT3 receptors, because the depolarization was mimicked by 2-methyl-5-HT and blocked by the 5-HT3 antagonist LY-278584. This type of response is thought to be indirect because it was blocked by atropine or TTX, indicating that both nerve conduction and the release of acetylcholine onto a muscarinic receptor led to the depolarization elicited by 5-HT. A TTX-sensitive 5-HT response has been previously reported in guinea pig submucosal neurons (25). The simplest explanation of these results is that microejection of 5-HT activates 5-HT3 receptors on a cholinergic interneuron, which subsequently releases acetylcholine onto a muscarinic receptor on the impaled SO neuron. Furthermore, in a subpopulation of SO neurons (~30%), both the indirect 5-HT depolarization and the fast depolarization to 5-HT were recorded in the same cell. This indicates that 5-HT3 receptors can be located on more than one neuron in a synaptically connected pathway of neurons.

The other response to 5-HT that was recorded in SO neurons was a prolonged depolarization that was not
modulated by 5-HT$_3$ receptors. The prolonged depolarization to 5-HT had time-course characteristics similar to the response of SO neurons to 5-HT superfusion. Although no experiments were performed to characterize the 5-HT superfusion response, it is speculated that this response was mediated through 5-HT receptor subtypes similar to those implicated in the prolonged depolarization to 5-HT pressure microejection.

In many other gut preparations, the prolonged depolarization evoked by 5-HT has been found to be mediated by the stimulation of 5-HT$_T$ receptors (14, 20, 21). In guinea pig SO neurons, the prolonged depolarization was significantly attenuated by renzapride but was not affected by 5-HT$_4$ receptor antagonism, demonstrating that the prolonged depolarization to 5-HT was largely mediated by 5-HT$_{1p}$ receptors. The prolonged depolarization was associated with an increase in input resistance. Slow EPSP (sEPSP) in enteric cell neurons are similarly associated with an increase in input resistance, and 5-HT is one of the many proposed mediators of sEPSP in the gut (12, 30). Thus the prolonged depolarization elicited by 5-HT in this study had similar characteristics to sEPSP evoked in SO neurons. Moreover, in preliminary experiments, evoked sEPSP were abolished in the presence of renzapride, suggesting that slow synaptic transmission can be mediated by 5-HT$_T$ receptors in the SO.

In summary, these data demonstrate that there is an extensive serotonergic innervation of the guinea pig SO, with a plethora of 5-HT-immunoreactive fibers but a relative sparsity of cell bodies. Most of the serotonergic immunoreactivity was intrinsic to the SO, because staining persisted in organ culture conditions. 5-HT elicits a variety of electrophysiological responses in SO neurons, including a fast depolarization that is mediated by 5-HT$_3$ receptors, a prolonged depolarization that mimics sEPSP and is mediated by 5-HT$_{1p}$ receptors, and an indirect effect on SO neurons that involves the stimulation of cholinergic neurons by a 5-HT$_3$ receptor. These results support the concept that neurally released 5-HT could play a physiological role in SO regulation through actions on SO neurons.

This work was supported by National Institutes of Health Grants NS-26995 and DK-45410 (to G. M. Mawe) and a Pharmaceutical Research and Manufacturers of America Foundation Fellowship (to K. Hilyad). Address for reprint requests: G. M. Mawe, C-423 Given Bldg., Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burlington, VT 05405.

Received 26 March 1998; accepted in final form 1 July 1998.

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