Roles of 5-HT receptors in the release and action of secretin on pancreatic secretion in rats

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Li, James P., Ta-Min Chang, and William Y. Chey. Roles of 5-HT receptors in the release and action of secretin on pancreatic secretion in rats. Am J Physiol Gastrointest Liver Physiol 280: G595–G602, 2001.—5-Hydroxytryptamine (serotonin, 5-HT) is a hormone and neurotransmitter regulating gastrointestinal functions. 5-HT receptors are widely distributed in gastrointestinal mucosa and the enteric nervous system. Duodenal acidification stimulates not only the release of both 5-HT and secretin but also pancreatic exocrine secretion. We investigated the effect of 5-HT receptor antagonists on the release of secretin and pancreatic secretion of water and bicarbonate induced by duodenal acidification in anesthetized rats. Both the 5-HT<sub>2</sub> receptor antagonist ketanserin and the 5-HT<sub>3</sub> receptor antagonist ondansetron at 1–100 μg/kg dose-dependently inhibited acid-induced increases in plasma secretin concentration and pancreatic exocrine secretion. Neither the 5-HT<sub>1</sub> receptor antagonists pindolol and 5-HTP-DP nor the 5-HT<sub>4</sub> receptor antagonist SDZ-205,557 affected acid-evoked release of secretin or pancreatic secretion. None of the 5-HT receptor antagonists affected basal pancreatic secretion or plasma secretin concentration. Ketanserin or ondansetron at 10 μg/kg or a combination of both suppressed the pancreatic secretion in response to intravenous secretin at 2.5 and 5 pmol·kg<sup>-1</sup>·h<sup>-1</sup> by 55–75%, but not at 10 pmol·kg<sup>-1</sup>·h<sup>-1</sup>. Atropine (50 μg/kg) significantly attenuated the inhibitory effect of ketanserin on pancreatic secretion but not on the release of secretin. These observations suggest that 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptors mediate duodenal acidification-induced release of secretin and pancreatic secretion of fluid and bicarbonate. Also, regulation of pancreatic exocrine secretion through 5-HT<sub>2</sub> receptors may involve a cholinergic pathway in the rat.

5-hydroxytryptamine; 5-hydroxytryptamine receptor; pancreatic exocrine secretion; atropine; rat

Although 5-hydroxytryptamine (5-HT) is widely distributed throughout the body, the largest store of 5-HT is found in the gastrointestinal tract. More than 90% of 5-HT is localized in the enterochromaffin (EC) cells of gastrointestinal mucosal epithelia and the enteric neurons. 5-HT is secreted from EC cells in response to a variety of luminal mechanical and chemical stimuli and activates neural circuits within the gastrointestinal wall. 5-HT is an important neurotransmitter and intercellular messenger to modulate gastrointestinal functions (14). 5-HT stimulates or inhibits gastrointestinal motility (8), alters gastric acid secretion (46), and enhances intestinal secretions (13, 56). Immunohistochemical study of gut mucosae (12) has demonstrated that EC cells together with other enteroendocrine cells have a morphological feature consistent with a paracrine action that may play a regulatory role in the release of gut hormones.

Through pharmacological studies and molecular cloning (1, 18, 42), at least seven families of 5-HT receptor subtypes, including 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>3A</sub>, 5-HT<sub>4A</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6A</sub>, and 5-HT<sub>7A</sub>, have been discovered. Each subtype of 5-HT receptor is involved in various regulatory functions in different organs (18). Kirchgessner et al. (26, 28) have demonstrated the presence of 5-HT<sub>1A</sub> and 5-HT<sub>1P</sub> receptors in the pancreas. 5-HT immunoreactivity was also found in pancreatic ganglia, acinar nerves, and glucagon-immunoreactive islet cells, which are innervated by serotonergic enteropancreatic axons (23, 27, 29). Recently, Li and Owyang (33) reported that both 5-HT<sub>2A</sub> and 5-HT<sub>3A</sub> receptors mediate peptone-stimulated pancreatic protein secretion that is mediated by a luminal CCK-releasing peptide in rats.

Secretin is a major gut hormone located in S cells of the upper small intestinal mucosa. Secretin is released from the upper small intestine in response to duodenal acidification. It stimulates pancreatic exocrine secretion in various animal species and humans. The action and release of secretin in response to luminal stimulation are mediated by neural and hormonal mechanisms (7, 44). The results of immunohistochemical studies have indicated that secretin- and 5-HT-containing cells coexist in the intestinal mucosa (40). Duodenal acidification stimulates the release of not only secretin but also 5-HT from the intestinal mucosa (41), but little is known about the role of 5-HT in regulation of release and action of secretin and pancreatic exocrine secretion.

The aim of the present study was to test the hypothesis that certain 5-HT receptor subtypes are involved in duodenal acidification-elicited release of secretin and in the action of secretin on pancreatic exocrine secretion.
secretion. Therefore, we determined the effect of antagonists of different 5-HT receptor subtypes on basal and acid-stimulated pancreatic exocrine secretion and the release of secretin in anesthetized rats. To gain further insight into the neural pathway involved with action of 5-HT receptors, atropine was administered in the rats treated with 5-HT receptor antagonists.

MATERIALS AND METHODS

Materials. Synthetic porcine secretin was a kind gift of Dr. David Coy (Tulane University, New Orleans, LA). Pindolol (a 5-HT1 receptor antagonist), ketanserin (a 5-HT2 receptor antagonist), and SDZ-205,557 (a 5-HT3 receptor antagonist) were purchased from Research Biochemicals (Natick, MA). N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP; a 5-HT1P receptor antagonist) was purchased from the Research Foundation for Mental Hygiene (New York, NY). Ondansetron (a 5-HT3 receptor antagonist) was purchased from Glaxo-Wellcome. Atropine was purchased from Genesee Pharmaceutical (Irvine, CA).

Animal preparation. Male Sprague-Dawley rats weighing 250–280 g were used in the experiments. The animals were housed under controlled temperature (23°C) and were fasted for 24 h with free access to tap water before surgery. Under anesthesia with 0.4 ml of 25% urethan/kg body wt administered intraperitoneally, a midline abdominal incision was made. A polyethylene tube (ID 3.0 mm, OD 4.0 mm) was inserted in the proximal duodenum 5 mm distal to the pylorus via the stomach followed by ligation of the pylorus for intraduodenal infusion of 0.03 N HCl or 0.15 M NaCl. A pancreatic duct cannula was made by inserting a PE-10 tube (ID 0.28 mm, OD 0.61 mm) at the junction between the pancreatic duct and the duodenal wall for collection of pancreatic juice. Another PE-10 tube was cannulated in the bile duct proximal to the pancreatic duct for diversion of bile to the exterior. The jugular vein was catheterized using a PE-50 tube (ID 0.58 mm, OD 0.96 mm). The tube was kept patent by infusing 0.15 M NaCl solution at a flow rate of 1 ml/h for injection of 5-HT receptor antagonists.

Experimental design. Experiments were started in each group of five rats 30 min after surgery. To study the effect of 5-HT receptors on basal pancreatic secretion, 0.15 M NaCl was infused intraduodenally at 4.5 ml/h for 90 min after 75 min of measurement of basal pancreatic secretion. At 60 min, vehicle or a 5-HT receptor antagonist (pindolol (1 mg/kg), 5-HTP-DP (1 mg/kg), ketanserin (0.1 mg/kg), ondansetron (0.1 mg/kg), or SDZ-205,557 (1 mg/kg)) was administered intravenously at 10 ml/h for 15 min before 0.15 M NaCl was infused. Pancreatic secretion of fluid and bicarbonate during the 90-min period after NaCl infusion was determined. To study the role of 5-HT receptors in endogenous secretin-stimulated pancreatic secretion by duodenal acidification, the receptor antagonist was injected intravenously 15 min before 0.03 N HCl was infused intraduodenally at 4.5 ml/h for 90 min. However, pindolol (0.1 mg/kg), 5-HTP-DP (0.5 mg/kg), SDZ-205,557 (1 mg/kg), or ketanserin or ondansetron (1, 5, 10, 50, and 100 μg/kg) was infused in this study. The doses of these antagonists were either used in previous studies (10, 33) or determined as effective in the present study.

To investigate the effect of 5-HT receptors on exogenous secretin-induced pancreatic secretion, synthetic porcine secretin in three stepwise increasing doses (2.5, 5, and 10 pmol·kg⁻¹·h⁻¹) was continuously administered intravenously for 60 min each after 1 h of basal pancreatic secretion was collected as a control group. In the other three groups of rats, ketanserin, ondansetron, or a combination of the two antagonists was administered intravenously at 10 μg/kg 15 min before exogenous secretin was administered.

Measurement. Pancreatic juice was collected continuously in a glass micropipette (Drummond Scientific, Broomall, PA, USA) in 30-min samples. The volume of pancreatic fluid was measured by calculating the length of pancreatic juice in the micropipette. Basal plasma secretin concentration was obtained by centrifugation and was stored at −70°C. Plasma secretin level was measured by a specific RIA as described previously (6, 45).

Statistical analysis. All data were calculated and expressed graphically as means ± SE. The volume and bicarbonate outputs of pancreatic fluid from the last 60-min samples during each treatment were compared with those from the 60-min samples in the basal period and were expressed as a percentage increase over the basal value. The effect of a 5-HT antagonist was then compared with that of the control during the basal state (0.15 M NaCl infused intraduodenally) or duodenal acidification. Statistical analysis of data was accomplished by one-way ANOVA. Tukey’s post hoc test was used for multiple comparisons. A value of P < 0.05 was considered statistically significant.

RESULTS

Effects of 5-HT receptor antagonists on basal pancreatic secretion. During the basal period, pancreatic exocrine secretion was stable with secretion of fluid and bicarbonate at 17.4 ± 3.2 μl/min and 0.54 ± 0.15 μeq/min, respectively. Neither 0.15 M NaCl infused intraduodenally nor any of the 5-HT receptor antagonists tested significantly influenced basal pancreatic secretion, although ketanserin at 100 μg/kg or SDZ-205,557 at 1 mg/kg slightly reduced pancreatic secretion (Table 1). Basal plasma secretin concentration was 1.8 ± 0.5 mU/mL and was not influenced by either 0.15 M NaCl or any of the 5-HT receptor antagonists tested (Table 1).

Effect of 5-HT receptor antagonists on acid-stimulated pancreatic secretion and release of secretin. Duodenal acidification with 0.03 N HCl significantly increased pancreatic secretion of fluid and bicarbonate by 78.2 ± 6.4 and 146.0 ± 26.4%, respectively, over the basal secretion and was significantly higher than that observed in the control rats infused intraduodenally with 0.15 M NaCl (Table 1). Concomitantly, plasma secretin level increased from 1.7 ± 0.4 to 5.9 ± 0.8 mU/mL. As shown in Table 2, pindolol, 5-HTP-DP, or SDZ-
Table 1. Effect of 5-HT antagonists on basal pancreatic exocrine secretion and the release of secretin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume, Δ%</th>
<th>HCO₃⁻ output, Δ%</th>
<th>Plasma Secretin, pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3.2 ± 2.0</td>
<td>5.4 ± 3.3</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>PD (1 mg/kg)</td>
<td>5.6 ± 3.4</td>
<td>11.0 ± 7.3</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>5-HTP-DP (1 mg/kg)</td>
<td>11.0 ± 7.3</td>
<td>6.7 ± 4.6</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>KT (0.1 mg/kg)</td>
<td>-5.2 ± 5.1</td>
<td>-1.8 ± 6.9</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>OD (0.1 mg/kg)</td>
<td>2.7 ± 3.4</td>
<td>2.3 ± 2.8</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>SDZ-205,557 (1 mg/kg)</td>
<td>-13.3 ± 5.7</td>
<td>-3.7 ± 6.0</td>
<td>1.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rats in each group. Δ%: percentage increase over basal values. PD, pindolol; 5-HTP-DP, N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide; KT, ketanserin; OD, ondansetron.

205,557 did not affect significantly the acid-stimulated increases in pancreatic secretion and plasma secretin concentration. In contrast, ketanserin and ondansetron dose-dependently inhibited pancreatic secretions of fluid and bicarbonate as well as the increase of plasma secretin concentration in response to duodenal acidification. Ketanserin produced maximal inhibitions of 85% in fluid (decreased to 11.4 ± 7.6% over basal secretion) and 82% in bicarbonate (decreased to 27.0 ± 10.1% over basal secretion) and a 56% decrease in plasma secretin concentration (decreased to 2.6 ± 0.2 pM; Fig. 1). Similarly, ondansetron produced maximal inhibitions of 82% in fluid (to 14.0 ± 7.6% over basal) and 82% in bicarbonate (to 26.0 ± 6.3% over basal) secretion and a 67.8% decrease in plasma secretin concentration (to 1.9 ± 0.2 pM; Fig. 2). Both ketanserin and ondansetron produced maximal inhibition at a dose of 50 μg/kg.

Effect of ketanserin and ondansetron on pancreatic secretion in response to exogenous secretin. Secretin at 2.5, 5, and 10 pmol·kg⁻¹·h⁻¹ iv dose-dependently increased pancreatic fluid and bicarbonate secretion (Fig. 3). The stimulatory effect observed with 5 pmol·kg⁻¹·h⁻¹ iv with an increase of 70.4 ± 11.9% and 123.9 ± 19.4% over basal secretion in volume and bicarbonate output, respectively, was similar to that observed with intraduodenal infusion of 0.03 N HCl. The effect of 10 pmol·kg⁻¹·h⁻¹ of secretin, on the other hand, with a 121.2 ± 9.8% increase in volume and 201.8 ± 25.6% in bicarbonate output over basal secretion was nearly two times as much as that stimulated by 0.03 N HCl. Both ketanserin and ondansetron at 10 μg/kg significantly suppressed pancreatic secretion in response to intravenous secretin at 2.5 and 5 pmol·kg⁻¹·h⁻¹ but not at 10 pmol·kg⁻¹·h⁻¹ (Fig. 3). For instance, ketanserin decreased volume and bicarbonate secretion stimulated by secretin at 5 pmol·kg⁻¹·h⁻¹ to 36.4 ± 10.1% and 67.7 ± 13.0% over basal secretion, respectively, while ondansetron decreased the values to 45.2 ± 6.4% and 76.5 ± 8.5%, respectively. On the other hand, pancreatic secretion in response to secretin at 10 pmol·kg⁻¹·h⁻¹ was not significantly affected by ketanserin (volume secretion: 105 ± 28%; bicarbonate: 194 ± 51% over basal) or by ondansetron (volume: 114 ± 29%; bicarbonate: 160 ± 26% over basal). When ketanserin and ondansetron were administered together, pancreatic secretion of volume and bicarbonate in response to secretin at 5 pmol·kg⁻¹·h⁻¹ was decreased to 32.6 ± 8.6% and 47.9 ± 5.9% over basal, respectively. However, the decrease was not significantly different from that produced by ketanserin or ondansetron alone. In contrast, pancreatic secretion in response to secretin at 10 pmol·kg⁻¹·h⁻¹ in the presence of both 5-HT receptor antagonists (122 ± 28 and 203 ± 16% over basal, respectively) was not significantly different from that observed with secretin alone. Similarly, the 5-HT₁ receptor antagonists pindolol and 5-HTP-DP did not affect pancreatic secretion stimulated by secretin given intravenously at all doses (data not shown). The 5-HT₄ receptor antagonist SDZ-205,557 also did not affect pancreatic secretion stimulated by all three doses of secretin. For instance, secretin at 5 pmol·kg⁻¹·h⁻¹ in the presence of SDZ-205,557 (1 mg/kg) produced an increase of fluid volume by 70.4 ± 4.2% and bicarbonate output by 129.8 ± 11.1% over basal secretion.

Effect of atropine on release of secretin and pancreatic secretion in response to ketanserin and ondansetron. Atropine at 50 μg/kg followed by 25 μg·kg⁻¹·h⁻¹ iv did not influence the increase in plasma secretin level in response to duodenal acidification. Atropine also did not affect inhibition of acid-evoked secretin release by intravenous administration of ketanserin or ondansetron at 10 μg/kg (Fig. 4, bottom). However, atropine at the same dose significantly reversed, albeit partially, the inhibition by ketanserin but not by ondansetron of pancreatic exocrine secretion during duodenal acidification (Fig. 4, top and middle). Similarly, atropine also partially reversed the inhibition by ketanserin of secretin (5 pmol·kg⁻¹·h⁻¹)-stimulated pancreatic fluid secretion by 52% (from 36.4 ± 10.4% to 54.1 ± 6.3% over basal, P < 0.05) and bicarbonate output by 64.5% (from 67.7 ± 13.0% to 104.0 ± 14.7%, P < 0.05; Fig. 5). Again, atropine did not influence ondansetron-inhibited pancreatic secretion in response to secretin (Fig. 5). In control experiments, neither the release of secretin nor pancreatic fluid and bicarbonate secretion in response to acid was significantly influenced by atropine (data not shown).

Table 2. Effect of 5-HT₁ and 5-HT₄ receptor antagonists on HCl-induced pancreatic secretion and plasma secretin level

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume, Δ%</th>
<th>HCO₃⁻ output, Δ%</th>
<th>Plasma Secretin, pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>78.2 ± 6.4</td>
<td>146.0 ± 26.4</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>HCl + PD</td>
<td>90.3 ± 15.6</td>
<td>115.7 ± 33.1</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>HCl + 5-HTP-DP</td>
<td>80.7 ± 20.2</td>
<td>130.5 ± 24.0</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>HCl + SDZ-205,559</td>
<td>68.5 ± 10.9</td>
<td>125.6 ± 25.4</td>
<td>4.9 ± 0.6</td>
</tr>
</tbody>
</table>

Values represent means ± SE for 5–6 rats in each group. Doses were as follows: HCl, 0.03 N HCl id; PD, 1 mg/kg iv; 5HTP-DP, 1 mg/kg iv; SDZ-205,559, 1 mg/kg iv. None of the 5-HT₁ and 5-HT₄ receptor antagonists significantly affected pancreatic secretion or secretin release evoked by duodenal acidification.
DISCUSSION

The results of the present study have demonstrated for the first time that both 5-HT2 and 5-HT3 receptors play significant roles in the mechanisms of the release and action of secretin on pancreatic exocrine secretion in response to duodenal acidification. We have observed that both the 5-HT2 receptor antagonist ketanserin and the 5-HT3 receptor antagonist ondansetron dose-dependently inhibit the duodenal acidification-elicited increase in pancreatic exocrine secretion of fluid and bicarbonate as well as elevation of plasma secretin concentration. These results suggest that both of these 5-HT receptor subtypes are involved in the release of secretin stimulated by duodenal acidification. We have also observed that these two antagonists inhibited pancreatic exocrine secretion in response to exogenous secretin at a dose up to 5 pmol·kg⁻¹·h⁻¹ but not at 10 pmol·kg⁻¹·h⁻¹. It has been shown previously that exogenous secretin at 5 pmol·kg⁻¹·h⁻¹ produces plasma secretin levels comparable to post-prandial plasma levels of secretin and thus is regarded as a physiological dose (6, 30). For the same reason, the dose of secretin higher than 5 pmol·kg⁻¹·h⁻¹, such as 10 pmol·kg⁻¹·h⁻¹ or higher, which raises plasma secretin levels much higher than that found after a meal, is regarded as a pharmacological dose. Therefore, it appears that both 5-HT2 and 5-HT3 receptors mediate only the physiological but not pharmacological action of secretin on the exocrine pancreas. However, the involvement of the 5-HT receptor appears to be receptor subtype selective, as the antagonists of 5-HT1 and 5-HT4 receptors had no effect on acid-evoked release of secretin or the action of exogenous secretin. It should be noted that the lack of an effect by the 5-HT4 receptor antagonist SDZ-205,557 suggested that the inhibitory effects of ondansetron mentioned above were not due to its weak interaction at the 5-HT3 receptor. These observations appear to parallel with the observation by Li and Owyang (33) that both 5-HT2 and 5-HT3 receptors are involved in peptone meal-elicited release of CCK-releasing peptide and CCK in the rat. The receptor subtype selectivity appears to vary in 5-HT-mediated secretion from rat small intestinal mucosa. The release of 5-HT from the EC cells is regulated by 5-HT3 receptors (37). Cholera toxin-induced mucin secretion was in part mediated only by 5-HT3 receptors in a capsaicin-sensitive pathway (38). On the other hand, 5-HT-stimulated fluid and electrolyte secretion involve 5-HT2, 5-HT3, and 5-HT4 receptors (36).

The mechanisms or pathways of 5-HT2 and 5-HT3 receptors that mediate acid-elicited release of secretin are not clear at present. Duodenal acidification is a potent luminal stimulation to evoke the release of both secretin (6) and 5-HT (41). It has been shown that the release of secretin by duodenal acidification is mediated through the release of a secretin-releasing peptide (SRP; see Ref. 31). In addition, the release of SRP and secretin by duodenal acidification is mediated through a vagal sensory afferent pathway (30). 5-HT is known to be present both in EC cells in the intestinal mucosa (11) and in enteric neurons (9). It is possible that 5-HT is released from EC cells as a paracrine messenger or...
perhaps, in part, from intrinsic neurons as a neurotransmitter in the gastrointestinal tract (14, 51). Thus, similar to its effects on fluid and electrolyte secretion (36), 5-HT as a paracrine messenger could act directly on 5-HT2 and 5-HT3 receptors in the membrane of cells containing SRP or S cells to trigger the release of secretin. So far, it remains unknown whether 5-HT receptors are present in these cells. Electrophysiological studies have identified the existence of 5-HT3 receptors in pre- and postganglionic autonomic neurons and in neurons of the sensory and enteric nervous system (18, 21, 51). In the rat, 5-HT3 receptor is detected in both submucosal and myenteric ganglia (19). Thus it is possible that 5-HT acting on the 5-HT3 receptor in sensory afferent neurons and nerve endings in submucous and myenteric plexuses of the enteric nervous system may trigger the release of SRP and/or secretin. The presence of 5-HT2 receptors in the enteric nervous system is not well documented. However, it has been reported that the 5-HT2 receptor mediates jejunal mesenteric afferent discharge in the rat (17). It is possible that the 5-HT2 receptor mediates acid-evoked secretin release through a similar mechanism. The mechanism or mechanisms through which 5-HT2 and 5-HT3 receptors mediate the action of secretin on pancreatic exocrine secretion are also unclear at present. However, these receptors appear to play significant roles only when secretin is endogenously released or given in a physiological dose. Thus the antagonist of both 5-HT2 and 5-HT3 receptors failed to inhibit pancreatic exocrine secretion when it was stimulated by a pharmacological dose such as 10 pmol·kg⁻¹·h⁻¹. This observation indicates that secretin at a pharmacological dose is able to act on the exocrine pancreas without mediation by the two 5-HT receptors. In a previous study (32) we observed that stimulation of pancreatic exocrine secretion by secretin in physiological doses was mediated through a capsaicin-sensitive ascending vagal sensory pathway. On the other hand, secretin in a pharmacological dose appeared to override the mediation via the ascending sensory pathway of the vagi. Hillsley and Grundy (16) reported that duodenal luminal perfusion with 150 mM HCl significantly increased mesenteric afferent nerve discharge that was not suppressed by a 5-HT3 receptor antagonist. Such a concentrated HCl solution is known to elevate plasma secretin above physiological concentrations (6, 30). Thus the electrical activity in the afferent nerve elicited by concentrated acid appeared to be a pharmacological effect and would not be affected by a 5-HT3 receptor antagonist in a similar fashion. It has been reported (51) that excitation of vagal afferent nerves by 5-HT in anesthetized rats is mediated by both 5-HT2 and 5-HT3 but not by 5-HT1 or 5-HT4 receptors. 5-HT was found to excite a large portion of afferent neurons in the dorsal motor nucleus.

Fig. 3. Effect of ketanserin (KT, 10 µg/kg) and ondansetron (OD, 10 µg/kg) on Sec-stimulated pancreatic secretion. KT, OD, or both antagonists were given to each group of rats. Values represent means ± SE for 5 rats in each group. *P < 0.05 compared with value for Sec alone. Both KT and OD inhibited pancreatic secretion stimulated by secretin at a physiological dose (2.5 or 5 pmol·kg⁻¹·h⁻¹) but not at a pharmacological dose (10 pmol·kg⁻¹·h⁻¹). The combination of the two antagonists produced a greater inhibition than their individual effect also at the physiological doses of Sec only.

Fig. 4. Effect of atropine (AT) on inhibition by KT and OD of acid-stimulated pancreatic secretion of fluid (top) and HCO₃⁻ (middle) and release of Sec (bottom). HCl, 0.03 N id; KT, 10 µg/kg iv; OD, 10 µg/kg iv; AT, 50 µg/kg followed by 25 µg·kg⁻¹·h⁻¹ iv. Values represent means ± SE from 5 or 6 rats in each group. AT partially reversed inhibition by KT but not by OD of acid-evoked pancreatic exocrine secretion. AT did not affect KT- or OD-induced inhibition of acid-evoked Sec release. NS, not significant.
of the vagus projecting to the duodenum and stomach in a ketanserin-sensitive manner (4). Both 5-HT2 and 5-HT3 receptor subtypes are also present in the dorsal vagal complex (50, 54) that supplies efferent fibers to the gut. Kirchgessner and Gershon (22) have demonstrated in the rat that vagal efferent fibers terminate predominantly in the myenteric plexus of the stomach and duodenum. The myenteric neurons receiving vagal efferent innervation are either 5-HT- or vasoactive intestinal peptide-containing neurons. These authors also demonstrated that the pancreas receives extrinsic innervation mainly from the myenteric plexus of the stomach and duodenum (23). Some of these neurons contain 5-HT and terminate mainly in pancreatic ganglia, some of them in the vicinity of acini, ducts, vessels, and islets. Thus, although both 5-HT2 and 5-HT3 receptors play significant roles in the ascending vagal sensory pathway-mediated pancreatic exocrine secretion, their sites of participation are not clear at present. It is possible that luminal acid also stimulates the release of 5-HT from both enteric and pancreatic serotonergic neurons, which in turn may potentiate the effect of secretin on ductular water and bicarbonate secretion by acting on 5-HT3 receptors. This mode of action by the 5-HT receptors is very similar to that reported by other investigators. Thus activation of 5-HT3 receptor by a selective 5-HT3 agonist, 2-methyl-5-HT, stimulated basal amylase secretion (24), whereas a 5-HT3 antagonist, ICS-205,930, inhibited peptone-induced pancreatic protein secretion (33). So far, 5-HT2 receptor has not been found in the pancreas, although it is widely distributed in the central nervous system (CNS) and peripheral tissues (3, 18). It is possible that a 5-HT2 receptor in sensory nerve fibers that connect to the vagal afferent pathway mediates the action of secretin. Alternatively, 5-HT2 and 5-HT3 receptors may be localized in different subset of vagal afferent nerves to mediate the action of secretin. This proposition of separate localization of 5-HT2 and 5-HT3 receptors appears to help explain the different sensitivity toward atropine between ketanserin- and ondansetron-induced inhibitions of secretin-stimulated pancreatic exocrine secretion. Moreover, because secretin-stimulated pancreatic exocrine secretion in the rat is not sensitive to atropine (32, 30), it is very likely that 5-HT2 receptor is involved in suppression of an inhibitory neural pathway that attenuates the pathway stimulated by secretin. Thus action of 5-HT on the 5-HT2 receptor may activate a cholinergic receptor-dependent pathway that would suppress a yet unidentified inhibitory neural pathway, thereby permitting activation of the vagal afferent pathway by secretin. On the other hand, antagonism with ketanserin at the 5-HT2 receptor would remove the suppression, in an atropine-sensitive fashion, of the inhibitory pathway that in turn inhibits the action of secretin. It should be noted that, although this hypothetical inhibitory neural pathway appears to provide a logical explanation of reversal by atropine of ketanserin-produced inhibition of secretin-stimulated pancreatic exocrine secretion, its existence remains to be elucidated. In addition, we cannot totally rule out that 5-HT2 receptor exists in the rat pancreas. These questions may be subjects of our future study.

It should be noted that the present study was carried out in anesthetized rats that have been shown to have plasma somatostatin concentration elevated to inhibit basal gastric acid secretion (47, 53). Varga et al. (47) have shown that the elevated somatostatin concentration in the circulation has no effect on basal pancreatic exocrine secretion. On the other hand, these authors have shown that CCK-stimulated protein secretion in anesthetized rats is enhanced by immunoneutralization with a specific anti-somatostatin monoclonal antibody. This observation indicated that endogenous somatostatin participates in modulation of CCK-stimulated exocrine secretion. Lucey et al. (35) have shown that duodenal acidification elevated plasma somatostatin concentration in humans. Thus it is potentially possible that endogenous somatostatin is involved in 5-HT2 and 5-HT3 receptor-mediated pancreatic exocrine secretion.

Singer et al. (43) have proposed the existence of an enteropancreatic reflex mechanism to regulate pancreatic enzyme secretion in the dog. Kirchgessner and coworkers have provided the anatomic (23, 25) and functional (23) evidence of such an enteropancreatic neural pathway in both the guinea pig and rat to participate in regulation of pancreatic enzyme secretion. The enteropancreatic reflex mechanism appears to involve both 5-HT1A and 5-HT1P receptor subtypes (23, 27). In addition, these receptors appeared to in-
volve an inhibitory pathway in the rat (24). In the present study, however, we found that neither pindolol nor 5-HTP-DP influenced basal or acid-induced pancreatic secretion of fluid and bicarbonate. It should be noted that, in the guinea pig, different mucosal stimulants have been shown to activate different subsets of submucosal neurons (28). Thus a similar enteropancreatic reflex mechanism may not be operating in acidic and secretin-stimulated ductal secretion of the rat pancreas. Alternatively, a similar reflex mechanism may exist without involving the 5-HT4 receptors. Nevertheless, whether 5-HT2 and 5-HT3 receptors are involved in the enteropancreatic reflex mechanism of pancreatic duct secretion remains unknown and requires further study.

The 5-HT4 receptor has been identified in the CNS and in a variety of tissues (3), including the vagus nerve (2). The presence of the 5-HT4 receptor in guinea pig enteric neurons (38, 48) and its function therein (2) have been documented. In the rat, the 5-HT4 receptor has been shown to mediate intestinal secretion (15, 36, 37) and motility (34, 52). In the present study, we were unable to observe that a 5-HT4 receptor-selective antagonist, SDZ 205,557 (5), influenced either basal or acid-stimulated release of secretin and exocrine pancreatic secretion of fluid and bicarbonate. This observation suggests that the 5-HT4 receptor may not participate in mediation of these functions. However, we cannot rule out that the absence of involvement by the 5-HT4 receptor in acid-induced secretin release and pancreatic exocrine secretion may be attributed to a species difference. It is probably worthwhile to test the role of 5-HT4 receptor in regulation of pancreatic exocrine secretion and the release of secretin in other species.

In summary, our results provide evidence for physiological roles of 5-HT2 and 5-HT3 receptors in the mechanisms of the release of secretin and pancreatic secretion of fluid and bicarbonate in response to duodenal acidification. Our data suggest that luminal acid in the duodenum may trigger EC cells to release 5-HT locally. 5-HT then activates 5-HT2 and 5-HT3 receptors in enteric submucosal and myenteric plexuses, which provide a neuronal pathway for the release of secretin to stimulate pancreatic exocrine secretion. Furthermore, the mechanism of pancreatic secretion of fluid and bicarbonate stimulated by exogenous secretin in a physiological dose or endogenous secretin probably involves 5-HT2 and 5-HT3 receptors in the vagal sensory afferent pathway.

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