Intestinal serotonin acts as paracrine substance to mediate pancreatic secretion stimulated by luminal factors

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POSTPRANDIAL PANCREATIC ENZYME secretion is mainly controlled by the hormone CCK and by the vagal-vagal reflex that activates cholinergic postganglionic neurons in the pancreas (30, 40, 45). Studies in rats (25, 28, 29) have shown that CCK at physiological levels stimulates pancreatic enzyme secretion via a capsaicin-sensitive vagal afferent pathway. We (26) recently studied the quantitative importance of CCK- and non-CCK-dependent luminal stimuli in the mediation of postprandial pancreatic secretion. Administration of L-364,718 inhibited 54% of pancreatic protein secretion evoked by intragastric administration of chopped rodent chow, suggesting that non-CCK-dependent pancreatic stimulants account for 46% of postprandial pancreatic secretion. A combination of L-364,718, 5-hydroxytryptamine subtype 3 (serotonin-3, 5-HT₃) antagonist ICS-205,930, and 5-HT₂ antagonist ketanserin inhibited 94% of pancreatic protein secretion induced by intragastric administration of chopped rodent chow (26). Previous studies (1, 4) have shown that 5-HT increases the discharge of vagal afferent fibers from the stomach and proximal intestine of the ferret. More recently, we (49) provided electrophysiological evidence that endogenously released 5-HT plays a major role in the signal transmission evoked by luminal factors to stimulate vagal nodose neurons. Intraluminal perfusion of 5-HT increased vagal afferent discharges in the same nodose neurons that were activated by luminal stimuli (49).

Several gastrointestinal hormones, including 5-HT, substance P, and CCK have been identified within the lumen of the gastrointestinal tract (36). Early studies (6, 7) indicated that increased luminal pressure evoked measurable increases in 5-HT from enterochromaffin (EC) cells in guinea pig ileum. Fujimiya and colleagues (12) recently provided morphological evidence that 5-HT normally stored in the secretory granules of EC cells is released into the cytoplasmic matrix and then diffused into the intestinal lumen in response to high intraluminal pressure. We hypothesized that 5-HT released from intestinal mucosal EC cells in response to luminal stimuli acts as a paracrine substance, activating mucosal vagal afferent fibers to stimulate pancreatic secretion. We demonstrated that luminally administered 5-HT activated mucosal vagal afferent fibers to stimulate pancreatic secretion. Studies using 5-HT...
receptor subtype antagonists characterized the 5-HT receptors involved in the mediation of pancreatic responses to luminal stimulation.

METHODS

Materials. Maltose, capsaicin, methscopolamine [(−)-scopolamine methyl nitrate, MSCP], and 5-HT (creatinine sulfate complex) were purchased from Sigma Chemical (St. Louis, MO). Ketanserin and CR-1409 were purchased from Research Biochemical International (Natick, MA). Ondansetron hydrocholoride was purchased from Glaxo Wellcome (Research Triangle Park, NC). N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophanamide (5-HTP-DP) was purchased from the Research Foundation for Mental Hygiene (New York, NY).

Animal preparation. All protocols used in this study were approved by the University of Michigan committee on use and care of animals. After an overnight fast, male Sprague-Dawley rats weighing 250–300 g were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body wt im, respectively). One-third of the initial dose of anesthetic was given every 90 min to maintain surgical anesthesia. One or more polyethylene catheters were placed in the external jugular veins for intravenous infusion with a syringe-driven pump. A polyethylene cannula (PE-10, Clay-Adams; Becton Dickinson, Sparks, MD) was inserted through a midline incision into the common bile-pancreatic duct at the sphincter of Oddi. To permit infusion of bile-pancreatic juice, a second cannula was placed into the duodenum, slightly above the sphincter of Oddi (Fig. 1). The abdominal wound was covered with saline-moistened gauze, and the rats were maintained at 37°C with a heating pad.

Pancreatic secretion study. After a 30-min stabilization period, the combined bile-pancreatic secretions were collected every 15 min. The volume was measured, and an aliquot was taken and diluted with distilled water for protein determination. The remaining undiluted bile-pancreatic juice was pumped back into the rat through the duodenal cannula during the next collection period at the rate of secretion of the preceding collection period. Protein in the bile-pancreatic juice was measured spectrophotometrically using the assay method of Bradford (5). Our previous study confirmed that the increase in protein output in the bile pancreatic juice after CCK-8 (32) and luminal non-CCK stimulation (unpublished observations) reflected the protein from the pancreatic source. Biliary juice protein did not increase with these stimulations.

Duodenal perfusion studies. A 20-cm segment of the small intestine, including the whole duodenum and proximal jejenum, was isolated between two cannulas positioned 4 (PE-60, 0.76-mm ID, 1.22-mm OD) and 24 cm (PE-190, 1.19-mm ID, 1.7-mm OD) from the pylorus. The distal cannula was left open throughout the experiment to avoid any increase in intraluminal pressure. The pylorus was ligated to prevent the reflux of intestinal test solution into the stomach. We (26) have previously shown that pancreatic secretion in response to intraduodenal perfusion of NaCl (500 mosmol/kgH2O) or maltose (300 mM) is blocked by 5-HT3 receptor antagonists. In the present study, we examined the effect of exogenous intraduodenal luminal application of 5-HT solution on pancreatic secretion. After a 45-min basal period, 5-HT (10−4, 10−5, or 10−6 M) was perfused into the duodenum at a constant rate of 3 ml/h by means of a peristaltic pump. Three test solutions were administrated separately for 75 min in random order, with a 45-min resting period between experiments to allow pancreatic secretion to return to basal levels. In a separate group of rats, a 20-cm length of ileum was isolated between two cannulas, and the pancreatic secretion in response to the luminal perfusion of 5-HT was measured as described in Pancreatic secretion study. Pancreatic secretory responses were reproducible in this anesthetized rat model. We (27, 31) have shown previously that after 5 h diversion of bile-pancreatic juice, intraduodenal infusion of peptone or CCK-releasing peptide caused significant increases of pancreatic protein output with an accompanying increase in plasma CCK levels.

Effects of MSCP or acute vagotomy on luminal 5-HT-stimulated pancreatic secretion. To evaluate the role of vagal cholinergic pathways in the mediation of pancreatic secretion in response to intraluminal 5-HT administration, a quaternary anti-muscarinic drug, MSCP, was dissolved in 0.9% NaCl and administered subcutaneously at a dose of 0.1 mg/kg, 30 min before the infusion of 5-HT. Vehicle solutions were given to the control animals. Acute vagotomy was performed on a separate group of rats. A midline incision was made in the abdominal wall, and the stomach was carefully manipulated to expose the esophagus and the gastric cardia. Both anterior and posterior trunks of the vagal nerves were transected. For the control experiments, the abdominal vagal nerves were exposed but not severed. Pancreatic secretion studies, as described previously (see Pancreatic secretion study), were performed 30 min after surgery.

Mucosal application of capsaicin. Vagal afferent fibers are classified into muscle and mucosal afferents. To investigate if mucosal afferent fibers are responsible for mediating pancreatic secretion evoked by luminal 5-HT, we examined the effects of intestinal mucosal application of capsaicin, which has been shown (19) to impair the neurotransmission of sensory fibers. After laparotomy, a 20-cm isolated segment of small intestine, including the duodenum and proximal jejenum, was temporarily ligated at both ends and filled with
2-ml capsaicin solution (6 mg/ml, dissolved in 10% Tween 80 in olive oil) (30). After 30 min, the capsaicin solution was removed by needle aspiration. Control animals were treated with vehicle dissolved in Tween 80 in olive oil. Intestinal perfusion studies were performed in the anesthetized rats 7 days after local capsaicin application. Rats were observed for normal eye-wiping movement to indicate that the local mucosal capsaicin treatment had no systemic effect. Briefly, a drop of capsaicin solution (0.1 mg/ml) dissolved in saline was instilled into the eye. Protective wiping movements with the forepaws indicated the absence of systemic effects due to capsaicin after the mucosal application. When a positive response was observed, the eye was immediately and thoroughly rinsed with water to keep the discomfort to a minimum. The stimulation was mild and brief, and there was no evidence of eye inflammation or any continued discomfort to the rats. In a previous study (30), we showed that this treatment completely abolished the pancreatic response to intraduodenal perfusion of maltose and hypertonic NaCl.

Perivagal capsaicin pretreatment in conscious rats. Physiological control of pancreatic secretion may be different in anesthetized and conscious rats. The anesthetic used in the present study was a mixture of xylazine and ketamine. The primary site of ketamine action appears to be the phencyclidine receptor on the N-methyl-D-aspartate receptor complex (18). Some phencyclidines have been shown to interact with the muscarinic receptors. Previous studies (18) have shown that ketamine may inhibit muscarinic signaling in the rat cortex and hippocampus. This effect may explain the significantly lower basal pancreatic protein output in anesthetized rats compared with conscious rats. Therefore, in the current study, to verify the physiological relevance of our observations in anesthetized rats, we evaluated the effects of perivagal capsaicin application on pancreatic secretion evoked by duodenal 5-HT stimulation in conscious rats. Before surgery, atropine was administrated (0.5 mg/kg ip) to reduce the acute effects of capsaicin on the cardiovascular and respiratory systems. After anesthesia with xylazine and ketamine, the abdominal vagal trunks were exposed. A small piece of gauze soaked in 1% capsaicin (0.2 ml/rat) was left on the vagal trunks for 30 min (28). Vehicle alone was applied to controls. The rats were allowed to recover, and a second operation was performed 7 days later to insert the bile-pancreatic duct and duodenal catheters. After anesthesia, a polyethylene catheter (PE-10) was inserted through a midline abdominal incision into the bile-pancreatic duct. A second catheter (PE-50) was placed in the duodenum slightly above the sphincter of Oddi for intestinal perfusion of bile-pancreatic juice. The catheters were brought through the body wall and pulled through a subcutaneous tunnel to an exit site between the scapulae. Disinfectant (chlorhexidine) was applied to prevent infection. A plastic jacket was attached to prevent the animal from touching the catheters. The cannulas were connected between experiments. In a separate group of rats, one catheter was placed into the external jugular vein for intravenous infusion of 5-HT antagonists with a syringe-driven pump. After recovery from anesthesia, the rats were returned to their home cages. Pancreatic secretion studies were performed 7 days later, after the rats had fully recovered from surgery. After an overnight fast, the rats were lightly restrained in Bollman cages and pancreatic secretion studies in response to intraduodenal perfusion of 5-HT solution were performed as described previously (see Duodenal perfusion studies). Bile-pancreatic juice was collected every 15 min. At the end of the experiment, rats were killed by decapitation under anesthesia.

Effect of CCK-A receptor antagonist CR-1409 on 5-HT-stimulated pancreatic secretion in conscious rats. To investigate if 5-HT in the duodenum stimulates pancreatic protein secretion through a CCK-dependent mechanism, we examined the effect of the CCK receptor antagonist CR-1409. In this study, CR-1409 (10 mg/kg) was dissolved in 0.005 N NaOH for intravenous administration. This dose has been shown to abolish the pancreatic response stimulated by a near-maximum dose of cerulein (38). After a 45-min basal period, intraduodenal 5-HT perfusion studies were performed as described in Duodenal perfusion studies.

Effect of 5-HT receptor antagonists on luminal 5-HT-stimulated pancreatic secretion in conscious rats. To characterize the 5-HT receptor subtypes involved in the mediation of luminal 5-HT-stimulated pancreatic secretion, the following receptor antagonists were used: the 5-HT1 receptor antagonist ondansetron (20 μg/kg ip) (47), the 5-HT2A receptor antagonist ketanserin (250 μg/kg iv) (35), and the 5-HT1p receptor antagonist 5-HTP-DE (1 mg/kg iv) (34). Pancreatic secretion responses to the luminal application of 5-HT were tested 30 min after the administration of each 5-HT receptor antagonist.

Measurement of 5-HT in blood and intestinal effluent perfusates. Intestinal perfusion was performed as described earlier. Maltose (300 mM), hypertonic NaCl (600 mosmol/kgH2O), and 10−3 M 5-HT (3.88 μg/ml) were each infused at 3 ml/h for 1 h. The effluent perfusates were kept on ice in test tubes containing Trasylol (2,500 IU/ml). The effluent volumes were measured to determine the 5-HT output. Blood from the portal vein was collected after the perfusion of each test solution. The samples were kept on ice in test tubes containing EDTA and Trasylol (2,500 IU/ml). Shortly after the completion of each experiment, 5-HT was extracted from aliquots of the perfusates and from the whole blood using 10% ZnSO4 and 1.0 N NaOH. Supernatants were stored at −20°C. Biochemical assays were performed using HPLC with a C18 column, and the elution of the compound was monitored by a fluorescence detector. The detection limit was 1 ng/ml, as described previously (44).

Peripheral serotonergic neuron destruction. To rule out the possibility that a neural source of 5-HT may contribute to the release of 5-HT evoked by luminal stimuli, we evaluated the effect of 5,7-dihydroxytryptamine (5,7-DHT), a specific neurotoxin that destroys neurons containing 5-HT, without affecting mucosal cells that contain 5-HT (16). 5,7-DHT does not cross the blood-brain barrier (14). However, this neurotoxin is taken up by noradrenergic nerve terminals. Administration of a norepinephrine uptake-inhibiting drug such as desipramine prevents this process and improves the selectivity of 5,7-DHT for serotonergic neurons (3). A group of rats was injected with 5,7-DHT (300 mg/kg ip) to deplete 5-HT from the peripheral (including enteric) neurons (16, 41). To protect the noradrenergic neurons from the neurotoxic effects, the rats were pretreated with desipramine (25 mg/kg ip) 60 min before the injection of 5,7-DHT. Intestinal perfusion studies were performed 7 days after 5,7-DHT administration.

Statistical analysis. Results were expressed as means ± SE. Multivariate ANOVA was used to evaluate the effects of repeated measurements over time, the effects of treatment, and the interaction between these two variables. Basal output was determined as the average of two 15-min periods. Cumulative output was calculated as the sum of protein output during the last 30 min of 5-HT infusion minus basal output. Zero cumulative output was identified as 100% inhibition. Subsequent comparisons were made with the Newman-Keuls test (InStat Biostatistics version 2.01, Graphpad.
RESULTS

Effects of intestinal luminal perfusion of 5-HT on pancreatic secretion. In the ketamine-xylazine anesthetized rat model, basal pancreatic secretion was stable, averaging 132 ± 12 mg/h (Fig. 2). Intraduodenal perfusion of 5-HT produced a dose-dependent increase in pancreatic protein secretion. Perfusion of 5-HT at 10^{-6}, 10^{-5}, and 10^{-4} at 3 ml/h increased protein secretion to 203 ± 6, 244 ± 8 and 312 ± 11 mg/h, which represented a 54%, 88%, and 140% increase over basal, respectively (Fig. 2A). These outputs were similar to those observed after intravenous infusion of CCK-8 at doses from 20 to 80 pmol·kg^{-1}·h^{-1} (28). In contrast, intraluminal infusion of 5-HT did not alter pancreatic protein output (Fig. 2A), indicating that intraluminal perfusion of 5-HT stimulates pancreatic secretion in a region-specific manner.

Effects of vagotomy, MSCP, and intestinal mucosal application of capsaicin. Neither intravenous administration of MSCP (a cholinergic muscarinic receptor antagonist that does not cross the blood-brain barrier) or acute vagotomy significantly affected basal pancreatic secretion in the anesthetized rat. However, these treatments completely abolished pancreatic responses to intraduodenal perfusion of 10^{-4} (Fig. 2B) and 10^{-5} M 5-HT (data not shown), indicating that luminal 5-HT acts on the vagal peripheral cholinergic pathway to stimulate pancreatic secretion. Furthermore, we showed that, in contrast to vehicle treatment, duodenal mucosal application of capsaicin completely abolished pancreatic secretion produced by intraduodenal perfusion of 5-HT (Fig. 2B), indicating that exogenous 5-HT acts on capsaicin-sensitive vagal sensory nerve endings near the lumen to stimulate pancreatic secretion.

Effects of perivagal application of capsaicin or CCK-A receptor antagonist CR-1409 on 5-HT-induced pancreatic secretion in conscious rats. Conscious rats have relatively high basal pancreatic secretion rates, averaging 308 ± 19 mg/h. Intraduodenal perfusion of 5-HT at concentrations of 10^{-5} and 10^{-4} M at 3 ml/h evoked 90% and 148% increases, respectively, in protein output over basal (from basal 308 ± 19 to 585 ± 16 or 763 ± 28 mg/h, respectively). Intravenous injection of the CCK-A receptor antagonist CR-1409 or perivagral application of capsaicin did not affect basal pancreatic enzyme secretion (Fig. 3). CR-1409 at a dose of 10 mg·kg^{-1}·h^{-1} did not affect the pancreatic response to intraluminal 5-HT infusion. These observations suggest that luminal 5-HT stimulates pancreatic enzyme secretion by a CCK-independent pathway. Similar to truncal vagotomy, perivagral application of capsaicin also completely abolished pancreatic secretion in response to luminal perfusion of 5-HT (Fig. 3). We (27) have previously shown that pancreatic protein secretion in response to 2-deoxyglucose stimulation remained intact in rats after perivagral application of capsaicin, indicating that this treatment does not affect efferent vagal function.

Effects of 5-HT receptor antagonists. In conscious rats, intraduodenal infusion of 10^{-5} M 5-HT increased pancreatic protein secretion from a basal level of 304 ± 16 mg/h to 569 ± 11 (Fig. 4A). Administration of ondansetron, a specific 5-HT3 antagonist, produced >90% inhibition of pancreatic secretion stimulated by intraduodenal administration of 5-HT (10^{-5} M). Administration of ketanserin, a specific 5-HT2A antagonist, did not produce a statistically significant inhibition of pancreatic secretion in response to 5-HT. 5-HT-stimulated pancreatic protein secretion was completely abolished by a combination of ondansetron and ketanserin (Fig. 4B). Intravenous administration of the 5-HT3 antagonist 5-HTP-DP did not affect luminal 5-HT-stimulated pancreatic secretion (Fig. 4A). These observations suggest that the 5-HT3 receptor subtypes located in mucosal vagal terminals are primarily involved in the mediation of pancreatic secretion stimulated by luminal application of 5-HT solution.

5-HT concentration in intestinal effluent perfusates and portal vein. Data on blood and intestinal perfusate 5-HT levels are presented in Fig. 5. In peripheral blood, basal 5-HT levels averaged 0.16 μg/ml. Neither maltose, hypertonic NaCl, or intraluminal perfusion of
Intraluminal perfusion of hypertonic NaCl (600 mosmol/kgH2O) or maltose (300 mM) produced a threefold increase in 5-HT concentrations in the intestinal effluent perfusates, from a basal level of 1.7 ± 0.2 to 5.2 ± 0.2 and 4.7 ± 1.1 μg/20 min, respectively. Intraluminal perfusion of 10^{-5} M 5-HT (3.88 μg/ml) at 3 ml/h resulted in a 5-HT level similar to that observed after intraluminal infusion of 600 mosmol/kgH2O NaCl. Intraperitoneal administration of 5,7-DHT had no effect on the luminal release of 5-HT evoked by luminal stimuli, indicating that a peripheral neuronal source of 5-HT was not responsible for increased 5-HT levels after luminal stimulation.

**Fig. 3.** In conscious rats, intraduodenal administration of 10^{-5} M 5-HT produced a 90% increase in pancreatic protein secretion (from basal 308 ± 19 to 585 ± 16 mg/h). Perivagal application of capsaicin abolished pancreatic secretion evoked by intraduodenal 5-HT. Pretreatment with the CCK-A receptor antagonist CR-1409 (10 mg/kg iv) had no effect on pancreatic protein secretion evoked by intraduodenal perfusion of 5-HT in conscious rats. *P < 0.05, capsaicin compared with vehicle treatment.

**DISCUSSION**

In mammals, significant levels of 5-HT exist in the EC cells of the gastrointestinal mucosa. In addition, there are serotonergic neurons in the intestinal myenteric plexus that may mediate local reflexes. However, the major source of 5-HT in the intestine appears to derive from the gastrointestinal mucosal EC cells, as the amount of 5-HT released from intestinal preparations with intact mucosa is 100-fold greater than the amount released from mucosa-free muscle preparations where serotonergic neurons preside (43). Large numbers of cells containing 5-HT exist in the proximal duodenum. The basal part of these cells rests on the basement membrane of the crypt epithelium while the apex often extends into the lumen (33). It was shown that the 5-HT is primarily stored in the EC cell secretory granules. The secretory granules are concentrated at the base of EC cells (12), suggesting that release at the basolateral membrane (i.e., interstitial side) is important. The morphology of these cells supports a paracrine sensory role (13). EC cells have been shown to secrete 5-HT spontaneously (43) and in response to a wide variety of stimuli (42). After stimulation, 5-HT is released across the basolateral membrane around afferent nerve terminals in the crypts and the villous of the lamina propria (48). A study by Berthoul et al. (2) showed that in the crypts and villus of rat duodenal mucosa vagal terminal branches came in close contact with the basal lamina but did not appear to penetrate it. It is these nerve endings that may well be the targets for the 5-HT released by the EC cells. In a recent electrophysiological study (49) of rat nodose ganglion, we demonstrated a neuronal response to intraduodenal perfusions of maltose, glucose, and hypertonic saline. These vagal primary afferent neurons were also sensitive to exogenous luminal 5-HT at concentrations that mimic physiological levels. Intravenous administration of a 5-HT{sub}3 antagonist blocked these responses, suggesting that nodose neu-
Intestinal serotonin stimulates pancreatic secretion

Fig. 5. A: luminal perfusion of maltose (300 mM) or hypertonic NaCl or exogenous infusion of 5-HT (10^{-5} M) did not significantly alter the concentration of 5-HT in the blood. B: perfusion of maltose or hypertonic NaCl evoked significant increases of 5-HT in the luminal perfusates, from 1.7 to 5–5.5 μg/20-min collection. Intraperitoneal administration of 5,7-dihydroxytryptamine (5,7-DHT), which destroyed peripheral neurons containing 5-HT, failed to affect the secretion of luminal 5-HT. The concentration of 5-HT in the luminal perfusates after luminal infusion of 5-HT (3.88 μg/ml) was 5.7 ± 1.7 μg/20-min collection. This was similar to that observed after intraluminal infusion of hypertonic NaCl. *P < 0.05 compared with basal level.

Exocrine cells in the digestive tract have been shown (23) to release their secretory granules from the basal cell membrane by exocytosis. A previous study (39) has shown a bilobed distribution of secretory granules in the EC cells of rat duodenum. Electron microscopy (12) of rat duodenal preparations provided morphological evidence to support the luminal release of 5-HT from the intestinal EC cell. In response to intraluminal pressure, 5-HT enters the extragranular matrix and is released into the lumen through the apical cell membrane. This process appears to be similar to that described for the release of gastrin from antral G cells after appropriate stimulation (11). In the present study, we examined 5-HT levels in peripheral blood and luminal effluent perfusates in response to luminal perfusion of maltose or hypertonic NaCl. We showed that luminal perfusion of maltose or hypertonic NaCl evoked the release of 5-HT from 1.7 to 5–5.5 μg per 20-min collection of luminal perfusates. Similar levels were observed after intraluminal infusion of 10^{-5} M 5-HT. In contrast, neither maltose, hypertonic NaCl, or intraluminal perfusion of 5-HT significantly altered 5-HT levels in portal venous blood. To determine the source of 5-HT responsible for increasing luminal 5-HT levels evoked by luminal stimuli, we evaluated the effect of 5,7-DHT, a specific neurotoxin known to destroy peripheral neurons that contain 5-HT without affecting mucosal EC cells that contain 5-HT (16). We found that peripheral administration of 5,7-DHT did not alter the increased luminal 5-HT levels evoked by luminal stimuli. Because we have ruled out involvement of the myenteric plexus, the most obvious source of 5-HT is the mucosal EC cells, which are well situated for sensing intraluminal chemical and mechanical events.

In a recent study (26), we demonstrated that in addition to CCK, non-CCK-dependent pancreatic stimulants accounted for 46% of pancreatic secretion and that 5-HT plays a critical role in mediating the non-CCK-stimulated pancreatic secretion in response to a chow meal in the rat. However, to date, there is no direct evidence that luminal 5-HT can stimulate pancreatic secretion via vagal afferent pathways. In the present study, we showed that the intraduodenal application of 5-HT (10^{-5}–10^{-6} M) stimulated pancreatic secretion in a dose-dependent manner. Luminal perfusion of 10^{-5} M 5-HT resulted in the 5-HT levels in effluent perfusates mimicking the 5-HT levels after intestinal perfusion of maltose or hyperosmolar saline. Similar luminal levels have been reported in studies (46) of the pyloric response to acid stimulation. In vitro studies (8) have demonstrated that intraluminally released 5-HT can diffuse through the gut wall and reach the neural plexuses in sufficient amounts to be physiologically important.

We next investigated the site of action of luminal 5-HT to stimulate pancreatic secretion. The ability of atropine and hexamethonium to completely abolish pancreatic enzyme responses to luminal application of 5-HT (data are not shown) suggests that 5-HT is acting at a presynaptic site along the cholinergic pathway. To identify the action site more accurately, we examined the effect of bilateral subdiaphragmatic vagotomy. Similar to atropine, vagotomy also completely abolished pancreatic responses. Our results therefore indicate that luminal 5-HT stimulates pancreatic secretion via the vagal cholinergic pathway. To investigate if 5-HT exerts its action via afferent or efferent vagal pathways, we examined the effect of perivagal treatment with the sensory neurotoxin capsaicin. Previous studies (28, 30) have demonstrated that this treatment abolishes pancreatic secretion stimulated by the vagal afferent pathways. In the current study, we showed that perivagal pretreatment with capsaicin impaired pancreatic responses to luminal 5-HT, an effect similar to that observed with vagotomy and with atropine. This indicates that the primary site of action of luminal 5-HT is the vagal afferent pathway. It is interesting to note that intraduodenal but not intraileal administra-
tion of 5-HT stimulated pancreatic secretion. This suggests that vagal afferent fibers terminating in the proximal but not the distal intestine are involved in the mediation of pancreatic secretion.

Retrograde and anterograde studies (20) have clearly demonstrated that there are neural fibers projecting from the gastric and intestinal myenteric plexuses to the pancreas. Furthermore, 5-HT-containing neurons in the myenteric plexus also possess 5-HT receptors. 5-HT receptors have also been identified on neural fibers located between the pancreatic acini and parenchyma (22). The physiological functions of these serotonergic receptors on enteropancreatic neural projections are not well understood. In isolated pancreatic lobules, 5-HT inhibited veratridine-evoked postganglionic cholinergic nerve-mediated amylase secretion (21). It should be noted that previous studies (9, 37) have shown that intravenous administration of 5-HT decreased pancreatic secretion in the dog and the rat. It is likely that 5-HT in the circulation may activate the enteropancreatic neural pathway to inhibit cholinergic transmission and reduce pancreatic secretion (21). We (24) have observed that serotonergic enteropancreatic innervations are responsible for inhibition of pancreatic secretion induced by duodenal distention. These inhibitory serotonergic pathways, however, should not be confused with the serotonergic receptors present in the submucous plexus, which appear to be responsible for activating the vagal afferent fibers terminating in the duodenal mucosa.

To characterize the 5-HT receptor subtypes involved in the mediation of pancreatic secretion evoked by luminal factors, we examined the effect of various 5-HT receptor antagonists: 5-HTP-DP, ondansetron, and ketanserin. The selective nature of these receptor antagonists has been demonstrated in previous studies (15, 17, 26, 34). We showed that administration of ondansetron markedly inhibited pancreatic secretion induced by intraduodenal perfusion of 5-HT, suggesting that 5-HT 

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Intestinal Serotonin Stimulates Pancreatic Secretion


