Significant cholinergic role in secretin-stimulated exocrine secretion in isolated rat pancreas

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Significant cholinergic role in secretin-stimulated exocrine secretion in isolated rat pancreas. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G413–G418, 1998.—Effects of intrapancreatic cholinergic activation by electrical field stimulation (EFS) on secretin-stimulated pancreatic exocrine secretion were investigated in the totally isolated perfused rat pancreas. EFS at 15 V, 2 ms, and 8 Hz for 45 min markedly increased spontaneous pancreatic secretion. This increase was completely inhibited by tetrodotoxin (1 µM) but not by hexamethonium (100 µM). Atropine (2 µM) significantly reduced the EFS-evoked volume flow and amylase output by 52% and 80%, respectively. EFS further increased the secretin (12 pM)-stimulated pancreatic secretion of fluid and amylase. The increases of the two parameters were significantly suppressed by atropine by 28% and 72%, respectively. Interestingly, EFS significantly increased concentrations of somatostatin-like immunoreactivity in portal venous effluents. When pertussis toxin (200 ng/ml) or rabbit antisomatostatin serum (0.1 ml/10 ml; titer of 1:50,000) was intra-arterially administered, EFS further increased the secretin-stimulated pancreatic secretion. In conclusion, the activation of intrapancreatic cholinergic neurons potentiated the secretin action on pancreatic exocrine secretion in the rat. This potentiating effect was significantly reduced by local somatostatin released during EFS that activated intrapancreatic cholinergic tone.

intrapancreatic cholinergic neurons; somatostatin; pancreatic exocrine secretion

The importance of the cholinergic system in secretin-stimulated pancreatic exocrine secretion has been recognized since the pancreatic secretory response to endogenous or exogenous secretin in a physiological dose was shown to be significantly reduced by vagotomy (5, 21) and atropine (5, 36). Moreover, atropine inhibits secretin-induced exocrine secretion from the intrinsically denervated pancreas in the dog (15, 21, 31) and the rat (3, 24). These reports suggest that suppression of basal intrapancreatic cholinergic tone results in a significant reduction of secretin-stimulated pancreatic exocrine secretion. However, there has been no direct evidence in the rat that an increased intrapancreatic cholinergic tone could influence the secretin-stimulated pancreatic exocrine secretion, although both vagal stimulation (5) and carbamylcholine (34), a cholinergic agonist, reportedly potentiate secretin-stimulated pancreatic exocrine secretion in the dog.

It has been documented that cholinergic activation by vagal stimulation or cholinergic agonists induces the release of somatostatin-like immunoreactivity in the dog (1, 7) and the rat (12). Since exogenous somatostatin inhibits the pancreatic exocrine secretion stimulated by secretin (8, 18, 30) as well as cholinergic agonists (4, 6, 32), it seems possible that effects of cholinergic activation on the secretin-stimulated pancreatic exocrine secretion are significantly influenced by concomitant release of somatostatin during the cholinergic activation.

In the present study, we have investigated to determine whether activation of intrapancreatic cholinergic neurons could affect the pancreatic exocrine secretion stimulated by secretin and whether somatostatin released locally influences the interaction between intrapancreatic cholinergic activation and secretin. An isolated perfused rat pancreas model was used in the study to eliminate possible influences of extrinsic nerves and circulating gut hormones other than secretin on pancreatic exocrine secretion.

MATERIALS AND METHODS

Experimental animal preparation. Male Sprague-Dawley rats, weighing 250–300 g, were anesthetized with an intraperitoneal injection of 25% urethan (Sigma, St. Louis, MO) at a dose of 0.7 ml/100 g body wt after 24-h fasting but with free access to water. They were killed by an intravenous overdose of urethan after isolation of the pancreas.

Preparation of totally isolated vascularly perfused pancreas. The isolated perfused pancreas was prepared according to the method described previously (25, 27). In brief, the abdominal aorta was carefully dissected and cannulated with polyethylene tubing (PE-50; Clay Adams, Parsippany, NJ), and then two separate ligatures were placed on the aorta above the celiac artery and below the superior mesenteric artery, respectively. The pancreatic duct was cannulated at its duodenal end with PE-10 tubing (Clay Adams). The portal vein was also cannulated with Tygon microbore tubing (Fisher Scientific, Pittsburgh, PA) to drain the perfusate. The pancreas was isolated with the duodenum but separated from neighboring organs and tissues. The isolated pancreas was perfused with modified Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg H2O) through the celiac and superior mesenteric arteries at a flow rate of 1.2 ml/min, using a multistaltic pump (Buchler, Kansas, MO). The perfusate contained 0.1% bovine serum albumin (Sigma), 3% dextran T-70 (Sigma), and 5.6 mM glucose (Sigma) and was continuously oxygenated with 95% O2 containing 5% CO2. The isolated pancreas was placed in a temperature-controlled chamber at 37°C, which was also continuously supplied with Krebs-Henseleit solution at a flow rate of 0.35 ml/min and oxygenated. After the 30-min equilibration period, pancreatic juice secreted in 15 min was sequentially collected throughout the entire period of the experiment. Fifteen-minute samples of portal effluent from 10 isolated pancreata were collected in ice-chilled test tubes to determine somatostatin-like immunoreactivity.
Effects of electrical field stimulation on spontaneous pancreatic exocrine secretion. Electrical field stimulation (EFS) was applied to the isolated perfused rat pancreas for 45 min, starting 60 min after the basal period, via a pair of coiled platinum electrodes immersed in the experimental chamber with a 5-cm distance. EFS was performed by using biphasic square waves with parameters of 15 V, 2 ms, and 8 Hz. Tetrodotoxin (Sigma), hexamethonium (Sigma), or atropine (Sigma) was dissolved in the perfusate at a concentration of 1, 100, or 2 µM, respectively. The perfusate containing individual drug was administered starting 60 min before EFS began, and the perfusion continued throughout the experiment.

Effects of EFS on secretin-stimulated pancreatic exocrine secretion. After the basal period, synthetic porcine secretin (Peninsula, Belmont, CA) at a concentration of 12 pM was intra-arterially infused into the isolated pancreas for 60 min. EFS was applied to the isolated pancreas starting 15 min after the secretin infusion began and was continued until the end of the experiment. Atropine was dissolved in the perfusate at a concentration of 2 µM starting 60 min before EFS began and was administered throughout the experiment.

**Fig. 1.** Effects of hexamethonium (HEX), tetrodotoxin (TTX), or atropine (ATR) on pancreatic secretion of fluid (volume flow, top) and amylase (bottom) evoked by electrical field stimulation (EFS). Left: time course of pancreatic exocrine secretion. Right: pancreatic exocrine secretion cumulated during period (45 min) of EFS. Each value represents mean ± SE of 6 pancreata. Tetrodotoxin and atropine, but not hexamethonium, significantly reduced (*P < 0.05) the EFS-evoked pancreatic secretion of fluid (volume flow) and amylase output.

**Fig. 2.** Effects of EFS on secretin (S)-induced pancreatic secretion of fluid (volume flow, top) and amylase (bottom). Left: time course of pancreatic exocrine secretion. Right: pancreatic exocrine secretion cumulated during period (45 min) of EFS. Each value represents mean ± SE of 6 pancreata. EFS significantly increased (*P < 0.05) the secretin-induced pancreatic volume flow and amylase output.

Pertussis toxin (Sigma), a well-known somatostatin inhibitor in pancreatic acinar cells (14, 35), was added to the perfusate at a concentration of 200 ng/ml and was administered throughout the entire experiment, starting 60 min before EFS began. A rabbit antisomatostatin serum with a titer of 1:50,000 (17) was also added to the perfusate at a concentration of 0.1 ml/10 ml starting 15 min before EFS began.

Measurements. Volume flow of pancreatic juice was determined by measuring the length of pancreatic juice collected in a microtube with a capacity of 3.8 µl/cm. α-Amylase activity in pancreatic juice was measured by the method of Rick and Stegbauer (28). Concentrations of somatostatin-like immunoreactivity in the 15-min samples of portal effluent were determined by radioimmunoassay (17).

Statistical analysis of data. All data are illustrated as means ± SE. The statistical analysis was evaluated by the Student's t-test. The difference was considered significant when P < 0.05.

**RESULTS**

Effect of EFS on spontaneous pancreatic exocrine secretion. During the basal period, the isolated perfused rat pancreata spontaneously secreted a minute...
an additive effect on the secretion of fluid and a potentiation effect on amylase secretion. Atropine significantly reduced \( P < 0.05 \) the stimulatory effects of EFS on the secretin-stimulated secretion of fluid and amylase by 28% and 72%, respectively (Fig. 3).

Role of endogenous somatostatin in the action of EFS on secretin-stimulated pancreatic exocrine secretion. EFS significantly increased \( P < 0.05 \) the mean concentration of somatostatin-like immunoreactivity in portal venous effluents, from the basal level of 0.90 ± 0.18 pM to the peak level of 1.94 ± 0.15 pM. When pertussis toxin was administered, the EFS-enhanced secretin-stimulated secretion of fluid and amylase was further increased to 29.98 ± 1.36 µl/45 min and 368.47 ± 32.54 U/45 min, respectively (Fig. 4), and these increases were statistically significant \( P < 0.05 \). However, pertussis toxin did not influence the pancreatic secretion induced by secretin alone (Fig. 5). Likewise, when

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Fig. 3. Effects of atropine (ATR) on action of EFS-potentiated secretin-induced pancreatic secretion of fluid (volume flow, top) and amylase (bottom). Left: time course of pancreatic exocrine secretion. Right: pancreatic exocrine secretion cumulated during the period (45 min) of EFS. Each value represents mean ± SE of 6 pancreata. Atropine significantly reduced \( *P < 0.05 \) the action of EFS on the secretin-induced pancreatic volume flow and amylase output.

Fig. 4. Effects of pertussis toxin (PTX) on the EFS-potentiated secretin (S)-induced pancreatic secretion of fluid (volume flow, top) and amylase (bottom). Left: time course of pancreatic exocrine secretion. Right: pancreatic exocrine secretion cumulated during the period (45 min) of EFS. Each value represents mean ± SE of 6 pancreata. Pertussis toxin significantly increased \( *P < 0.05 \) the action of EFS on the secretin-induced pancreatic volume flow and amylase output.
antisomatostatin serum was intra-arterially infused (Fig. 6), the EFS-enhanced secretin-stimulated fluid and amylase secretion significantly increased to 27.46 ± 0.50 µl/45 min and 400.50 ± 31.59 U/45 min, respectively (*P < 0.05). Thus, when the action of endogenous somatostatin was blocked by either pertussis toxin or antisomatostatin antibody, the EFS-enhanced secretin-stimulated pancreatic secretion of fluid as well as amylase was further increased.

**DISCUSSION**

In the present study, EFS of the isolated perfused rat pancreas resulted in a marked increase in spontaneous exocrine secretion, including flow volume and amylase output. The EFS-evoked pancreatic secretion of both fluid and amylase was completely blocked by tetrodotoxin and partially inhibited by atropine. This supports previous reports (26, 33) that EFS-evoked amylase secretion was significantly inhibited by atropine in isolated segments of the rat pancreas. These results indicate that the effect of EFS on pancreatic exocrine secretion is neurally mediated and that EFS exerts cholinergic activation. Extrinsic nerves that may be present in the isolated pancreas do not appear to participate in the action of EFS, because hexamethonium, a ganglionic blocker, did not influence the pancreatic secretory response to EFS in the present study, although it completely blocked vagally stimulated pancreatic secretion in other studies in the rat (9) and the pig (20). Thus, in the isolated rat pancreas model we used, EFS mainly activated the intrapancreatic postganglionic cholinergic neurons to increase pancreatic secretion, in particular, amylase secretion. More significant reduction by atropine in amylase output than in volume flow suggests that spontaneous pancreatic amylase secretion is more sensitive to the intrapancreatic cholinergic activation than secretion of fluid. The incom-
complete inhibition by atropine of the EFS-evoked rat pancreatic exocrine secretion suggests that noncholinergic excitatory neurons, including peptidergic excitatory neurons (2, 19) as well as serotoninergic neurons (13), may also be activated by EFS. The possible role of intrapancreatic noncholinergic excitatory neurons in the EFS-evoked pancreatic exocrine secretion remains to be elucidated in future studies. It has been recently shown that vagal stimulation releases several neuropeptides in the rat that are known to stimulate pancreatic secretion. These include cholecystokinin, vasoactive intestinal peptide, and pituitary adenylate cyclase-activating peptide (16).

Although vagal stimulation (5, 11) and carbamylcholine (34), a cholinergic agonist, potentiate secretin-stimulated pancreatic exocrine secretion in the dog, there has been no direct evidence reported that activation of intrapancreatic cholinergic neurons affects secretin-induced pancreatic secretion. In this study, we have shown that EFS of the isolated rat pancreas further increased the secretin-stimulated flow volume and amylase output. Both an additive effect on flow volume and a potentiation effect on amylase output were observed. The results suggest that EFS exerted a stimulatory influence on secretin-stimulated pancreatic exocrine secretion by activating in part the intrapancreatic cholinergic neurons. The secretin-stimulated amylase output appeared more sensitive to the intrapancreatic cholinergic activation than the fluid secretion stimulated by secretin. Atropine was shown previously to inhibit the secretin-stimulated exocrine secretion in the denervated pancreas in rats (3) and dogs (15, 21, 31). These results and the present study suggest that the pancreatic secretory response to secretin is greatly dependent on the intrapancreatic cholinergic tone.

It has been reported that vagal stimulation increases somatostatin release, which was blocked by atropine in the dog (1, 7), although controversial results were reported in studies in the pig (10) and in the rat (22). However, acetylcholine has been documented to induce somatostatin release in the rat (12). Since somatostatin is a well-known islet hormone that suppresses pancreatic exocrine secretion stimulated by secretin (18, 30) or cholinergic stimulation (4, 6, 32), we suggest that the stimulatory effect of EFS on pancreatic exocrine secretion is influenced by locally released somatostatin that was concomitantly released along with acetylcholine. The level of somatostatin in portal venous effluent was significantly higher than control in the present study, which reflects local release of somatostatin. The local concentration of somatostatin in portal venous effluent was significantly higher than that in portal venous effluent. To test this hypothesis, we blocked the action of endogenous somatostatin by using pertussis toxin (14, 35). When pertussis toxin was intra-arterially administered into the isolated perfused rat pancreas, EFS further increased the secretin-stimulated secretion of fluid and amylase, to levels significantly higher than that of control. Thus the enhancing effect of EFS on the secretin-stimulated pancreatic secretion of fluid and amylase was significantly greater when the action of endogenous somatostatin was eliminated. Although pertussis toxin increased the EFS-evoked pancreatic exocrine secretion, it did not affect the secretin-stimulated pancreatic exocrine secretion. A similar result was obtained when a somatostatin antiserum was given to immunoneutralize endogenous somatostatin. The results provide evidence that endogenous somatostatin released by EFS exerts an inhibitory influence on the interaction between intrapancreatic cholinergic activation and secretin on pancreatic exocrine secretion. The failure of pertussis toxin in enhancing the pancreatic secretion stimulated by secretin alone suggests that secretin-stimulated pancreatic secretion without EFS is not influenced by locally available somatostatin. It is quite possible that without EFS, local concentration is significantly lower than that released during EFS.

Our observations on the release of somatostatin by EFS in the pancreas and the effect of antisomatostatin serum, however, are at variance with other reports (20, 22). Nishi et al. (22) reported that vagal stimulation inhibited somatostatin release from the isolated perfused rat pancreas; however, their isolated pancreas preparation included the stomach. Since somatostatin release from the stomach is reportedly inhibited by vagal stimulation (23, 29), it is possible that the concentration of somatostatin in portal venous effluent of their preparation (22) could have shown inhibition, reflecting mainly the inhibitory effect of somatostatin release. Nelson et al. (20) observed in anesthetized rats that immunoneutralization of endogenous somatostatin failed to alter the pancreatic amylase secretion stimulated by vagal stimulation. However, this discrepancy remains to be explained, because characteristics of the two antisomatostatin antibodies have not been compared.

In conclusion, activation of the intrapancreatic cholinergic neurons exerts an enhancing effect on secretin-stimulated pancreatic secretion of fluid and amylase. This cholinergically stimulated pancreatic secretion is under an inhibitory control of locally released somatostatin by EFS.

We thank Patricia Faiello for manuscript preparation.

This study was supported by Non-Directed Research Fund, Korea Research Foundation, 1996; and by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-25962.

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Received 23 June 1997; accepted in final form 13 November 1997.

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