Effects of $\gamma$-aminobutyric acid on secretagogue-induced exocrine secretion of isolated, perfused rat pancreas

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Park, Hyung Seo, and Hyoung Jin Park. Effects of $\gamma$-aminobutyric acid on secretagogue-induced exocrine secretion of isolated, perfused rat pancreas. Am J Physiol Gastrointest Liver Physiol 279: G677–G682, 2000.—Because GABA and its related enzymes have been determined in $\beta$-cells of pancreas islets, effects of GABA on pancreatic exocrine secretion were investigated in the isolated, perfused rat pancreas. GABA, given intra-arterially at concentrations of 3, 10, 30, and 100 $\mu$M, did not exert any influence on spontaneous or secretin (12 $\mu$M)-induced pancreatic exocrine secretion. However, GABA further enhanced CCK (10 $\mu$M)-, gastrin-releasing peptide (100 $\mu$M)-, or electrical field stimulation-induced pancreatic secretions of fluid and amylase dose dependently. The GABA (30 $\mu$M)-enhanced CCK-induced pancreatic secretions were completely blocked by bicuculline (10 $\mu$M), a GABA $\alpha$ receptor antagonist, but were not affected by saclofen (10 $\mu$M), a GABA $\delta$ receptor antagonist. The enhancing effects of GABA (30 $\mu$M) on CCK-induced pancreatic secretions were not changed by tetrodotoxin (1 $\mu$M) but were partially reduced by cyclo-(7-aminoheptanonyl-Phe-D-Trp-Lys-Thr[BZL]) (10 nM), a somatostatin antagonist. In conclusion, GABA enhances pancreatic exocrine secretion induced by secretagogues, which predominantly induce enzyme secretion, via GABA $\alpha$ receptors in the rat pancreas. The enhancing effect of GABA is partially mediated by inhibition of islet somatostatin release.

gamma-aminobutyric acid receptor; cholecystokinin; secretin; gastrin-releasing peptide

IT HAS BEEN REPORTED THAT GABA, a well-known inhibitory neurotransmitter, is contained in islet $\beta$-cells of the pancreas at a high concentration comparable to that in the brain (10, 20). Glutamate decarboxylase (GAD), a GABA-synthesizing enzyme, and GABA transaminase, a GABA-metabolizing enzyme, have also been found in $\beta$-cells (19, 26, 31, 34). However, the physiological role of endogenous GABA synthesized from $\beta$-cells in pancreatic function is obscure at present.

GABA appears to have some influence on pancreatic endocrine function. It has been reported that GABA concentration and GAD activity are very high in insulinoma tissue (20). GABA content in the endocrine pancreas is markedly decreased by streptozotocin, a selective $\beta$-cell toxin (13, 32, 34). GAD is associated with insulin-dependent diabetes mellitus (1). GABA or its agonist inhibits release of somatostatin (29, 32) and glucagon (30), whereas it stimulates release of insulin (12).

An influence of GABA on pancreatic exocrine function is completely unknown at the present time, although some reports suggest this possibility. High-affinity binding sites of GABA have been determined in pancreatic intralobular ductal cells and centroacinar cells (27). GABA immunoreactivity has been detected in zymogen granules of the rat pancreas (8). In addition, there is good evidence suggesting that GABA may be released from pancreatic $\beta$-cells. It has been reported that $\beta$-cells have synaptic-like microvesicles that may be concerned with GABA secretion (26) and that GABA is released from the $\beta$-TC6 pancreatic cell line (11). Thus it is assumed that GABA released from $\beta$-cells may play a role in pancreatic exocrine secretion through the islet-acinar portal system (15, 18).

Therefore, the present study was undertaken to investigate whether GABA can affect exocrine secretion in the isolated, perfused rat pancreas. Neural and hormonal mechanisms as well as a subtype of the GABA receptor associated with GABA activity in the exocrine pancreas were also determined.

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 a 24-h fast with free access to water. Rats were killed by an intravenous overdose injection of urethan after isolation of the pancreas.

Preparation of totally isolated and vascularly perfused pancreas. The isolated, perfused rat pancreas was prepared according to a method described previously (21, 25). In brief, the abdominal aorta was carefully dissected and cannulated with PE-50 tubing (Clay-Adams, Parsippany, NJ) just above the celiac axis and then tightly ligated just below the superior mesenteric artery. The pancreatic duct was cannulated at the duodenal end with PE-10 tubing (Clay-Adams). The
Effects of GABA on secretagogue-induced pancreatic exocrine secretion. Exocrine secretion of the isolated pancreas was stimulated by intra-arterial infusion of synthetic porcine secretin (Peninsula, Belmont, CA) at a concentration of 12 pM, synthetic sulfated CCK-8 (Squibb Institute, Princeton, NJ) at a concentration of 10 pM, or synthetic porcine gastrin-releasing peptide (GRP; Peninsula) at a concentration of 100 pM for 60 min. GABA (Sigma) at a concentration of 3, 10, 30, or 300 μM was added to perfusate from 45 min before the secretagogue infusion until the end of the experiment.

GABA, at all concentrations used, did not change the spontaneous pancreatic secretions or secretin (12 pM)-induced pancreatic secretion. CCK-8 (10 pM) significantly increased the basal pancreatic secretions of fluid and amylase to levels of 37.39 ± 3.70 U/60 min and 1.47 ± 0.29 μl/60 min, respectively, which were also further dose-dependently elevated by GABA. GABA, at a concentration of 30 μM, maximally enhanced the CCK-induced pancreatic secretions of fluid and amylase to levels of 38.91 ± 4.83 μl/60 min (P < 0.01) and 764.64 ± 93.49 U/60 min (P < 0.01), respectively.

Effects of GABA on electrical field stimulation-induced pancreatic exocrine secretion. Intrapancreatic neurons were excited by application of electrical field stimulation (EFS) using biphasic square waves (15 V, 2 ms, 8 Hz) for 45 min as described previously (23, 24). GABA at a concentration of 3, 10, 30, or 100 μM was added to the perfusate from 45 min before EFS until the end of the experiment.

Effects of GABA on EFS-induced pancreatic exocrine secretion. Figure 2 shows effects of GABA on pancreatic exocrine secretion induced by excitation of intrapancreatic neurons by EFS. EFS significantly increased pancreatic secretions of fluid and amylase from the basal levels to 16.16 ± 0.53 μl/45 min (P < 0.001) and 237.21 ± 19.61 U/45 min (P < 0.001), respectively. GABA, at concentrations of 3, 10, 30, and 100 μM, further dose-dependently elevated the EFS-induced pancreatic exocrine secretion.
Effects of GABA receptor antagonists on GABA action in CCK-induced pancreatic exocrine secretion. As shown in Fig. 3, GABA at a concentration of 30 μM markedly increased CCK-induced pancreatic volume flow and amylase output by 52.1% and 120.5%, respectively. Bicuculline at a concentration of 10 μM significantly reduced the GABA-enhanced CCK-induced pancreatic secretions of fluid and amylase by 36.4% (P < 0.01) and 49.4% (P < 0.05), respectively. Saclofen at a concentration of 10 μM failed to change the enhancing effect of GABA on the CCK-induced pancreatic exocrine secretion. Both bicuculline and saclofen exerted no influence on pancreatic exocrine secretion induced by CCK alone.

Effects of tetrodotoxin on GABA action in CCK-induced pancreatic exocrine secretion. Figure 4 illustrates effects of inhibition of intrapancreatic neuronal activity on GABA action. Tetrodotoxin at a concentration of 1 μM significantly reduced the CCK-induced pancreatic secretions of fluid and amylase to 14.39 ± 1.20 μl/60 min (P < 0.001) and 202.75 ± 27.99 U/60 min (P < 0.01), respectively. After tetrodotoxin, GABA (30 μM) still significantly elevated the CCK-induced secretions of fluid and amylase by 10.22 ± 0.33. However, the incremental rates of fluid and amylase secretion (51.6% and 114.1%, respectively) with tetrodotoxin were very similar to those (52.1% for fluid and 120.5% for amylase, respectively) without tetrodotoxin.

Effects of a somatostatin antagonist on GABA action in CCK-induced pancreatic exocrine secretion. Figure 5 shows effects of inhibition of endogenous somatostatin action on the action of GABA. Cyclo-(7-aminohexanoyl-Phe-δ-Trp-Lys-Thr(BZL)), a somatostatin antagonist, at a concentration of 10 nM significantly increased (P < 0.05) the CCK-induced pancreatic secretions of fluid and amylase by 20.2% and 43.2%, respectively. In the presence of the somatostatin antagonist, GABA still significantly increased (P < 0.05) the CCK-induced pancreatic secretions of fluid and amylase by 19.6% and 50.9%, respectively. However, the incremental rates of fluid and amylase secretion

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**Fig. 2.** Effects of GABA on electric field stimulation (EFS)-induced secretions of fluid (A) and amylase (B) in the isolated, perfused rat pancreas. Each value represents mean ± SE of 7 pancreata. *Significant differences (P < 0.05) compared with corresponding value obtained without GABA. GABA dose-dependently enhanced the EFS-induced pancreatic secretions of fluid and amylase.

**Fig. 3.** Effects of GABA antagonists on GABA-enhanced CCK-induced secretions of fluid (A) and amylase (B) in the isolated, perfused rat pancreas. Each value represents mean ± SE of 7 pancreata. Bicuculline (10 μM) completely inhibited the GABA (30 μM)-enhanced CCK (10 pM)-induced pancreatic secretion, but saclofen (10 μM) failed to change it. ND, no significant difference.

**Fig. 4.** Effects of TTX on CCK-induced secretions of fluid (A) and amylase (B) in the isolated, perfused rat pancreas. Each value represents mean ± SE of 7 pancreata. GABA (30 μM) enhanced the CCK (10 pM)-induced pancreatic secretion even in the presence of TTX (1 μM). The incremental rates are identical to those obtained without TTX.

**Fig. 5.** Effects of somatostatin antagonist on GABA action in CCK-induced pancreatic exocrine secretion. Figure 5 shows effects of inhibition of endogenous somatostatin action on the action of GABA. Cyclo-(7-aminohexanoyl-Phe-δ-Trp-Lys-Thr(BZL)), a somatostatin antagonist, at a concentration of 10 nM significantly increased (P < 0.05) the CCK-induced pancreatic secretions of fluid and amylase by 20.2% and 43.2%, respectively. In the presence of the somatostatin antagonist, GABA still significantly increased (P < 0.05) the CCK-induced pancreatic secretions of fluid and amylase by 19.6% and 50.9%, respectively. However, the incremental rates of fluid and amylase secretion
neurons participating in neuron-mediated pancreatic exocrine secretion in the rat. Because acetylcholine and GRP are neurotransmitters predominantly stimulating pancreatic enzyme secretion (4, 7, 16), effects of GABA on pancreatic exocrine secretion induced by intrapancreatic neuronal excitation were also investigated in this study. GABA dose-dependently increased pancreatic secretions of fluid and amylase in this study. GABA does not modify pancreatic secretions of fluid and amylase in this study. Thus GABA appears to exert the enhancing effect on CCK-induced pancreatic exocrine secretion via the GABA_A receptor in the rat pancreas. Because intrapancreatic neurons play a stimulatory role in CCK-induced pancreatic exocrine secretion (24), this study investigated whether intrapancreatic neurons mediate the GABA action. When tetrodotoxin (1 μM) was administered into the isolated rat pancreas, the enhancing effect of GABA on CCK-induced pancreatic secretions of fluid and amylase was reduced by ~50%. The result seems to suggest that the GABA action may be tetrodotoxin-sensitive. However, the incremental rates of CCK-induced fluid and amylase secretions (51.6% and 114.1%, respectively) caused by GABA with tetrodotoxin were very similar to the rates (52.1% for fluid and 120.5% for amylase, respectively) obtained without tetrodotoxin. This result also suggests that GABA may enhance the CCK-induced pancreatic secretion in a tetrodotoxin-insensitive manner. We showed previously (23) that 1 μM of tetrodotoxin completely inhibits EFS-induced secretions of fluid and amylase of the isolated, perfused rat pancreas. Thus it is suggested that the basal activity of the intrapancreatic neuron is essential to the action of GABA but GABA does not modify neuronal activity to elevate CCK-induced pancreatic exocrine secretion.

It has been reported that GABA and its agonist reduce somatostatin release from pancreatic islets (29, 32). Because somatostatin inhibits CCK-induced pancreatic exocrine secretion (9, 24), this study also examined whether GABA elevates CCK-induced pancreatic exocrine secretion by inhibition of release of endoge-
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nous somatostatin. When the action of endogenous somatostatin was blocked by cyclo-(7-aminopen- 
tonanyl-Phe-d-Trp-Lys-Thr[BZL]), a somatostatin antagonist (6, 24), pancreatic exocrine secretion induced by CCK alone was further elevated but the secretion induced by simultaneous GABA and CCK was not changed. However, in the presence of the somatostatin antagonist, GABA elevated the CCK-induced pancreatic secretions of fluid and amylase by 19.6% and 50.9%, respectively. The incremental rates were less than one-half of the rates (52.1% and 120.5%, respectively) obtained without the somatostatin antagonist. The results suggest that GABA may elevate the CCK-induced pancreatic exocrine secretion in part by inhibition of somatostatin release. The inhibition of somato- 
tostatin release by GABA in the rat pancreas remains to be elucidated in future studies.

In conclusion, GABA enhances pancreatic exocrine secretion induced by secretagogues, which predominantly stimulate enzyme secretion, via the GABA receptor in the rat pancreas. The enhancing effect of GABA is partially mediated by inhibition of islet soma-
tostatin release. The GABA action is dependent on the basal activity of the intrapancreatic neuron, but GABA does not modify the neuronal activity.

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