Effects of γ-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 γ-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 β-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 μM, did not exert any influence on spontaneous or secretin (12 pM)-induced pancreatic exocrine secretion. However, GABA further elevated CCK (10 μM)-, gastrin-releasing peptide (100 pM)-, or electrical field stimulation-induced pancreatic secretions of fluid and amylase dose dependently. The GABA (30 μM)-enhanced CCK-induced pancreatic secretions were completely blocked by bicuculline (10 μM), a GABA receptor antagonist, but were not affected by saclofen (10 μM), a GABA receptor antagonist. The enhancing effects of GABA (30 μM) on CCK-induced pancreatic secretions were not changed by tetrodotoxin (1 μM) but were partially reduced by cyclo-(7-aminooctanoyl-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 receptors in the rat pancreas. The enhancing effect of GABA is partially mediated by inhibition of islet somatostatin release.

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

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 vasculally 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 costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
hepatic portal vein was also cannulated with Tygon microbore tubing (Fisher Scientific, Pittsburgh, PA) to drain perfusate. The isolated pancreas was perfused with modified Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg H$_2$O) through the celiac axis and the superior mesenteric artery at a flow rate of 1.2 ml/min using a multistaltic pump (Buchler, Kansas City, MO). The perfusate contained 0.1% bovine serum albumin (Sigma), 5% Dextran T-70 (Sigma), and 18 mM glucose (Sigma) and was continuously oxygenated with 95% O$_2$-5% CO$_2$. The pancreas was isolated with the duodenum but separated from other neighboring organs and tissues and then placed in a temperature-controlled experimental chamber at 37°C. It was also continuously supplied with Krebs-Henseleit solution at a flow rate of 0.35 ml/min and oxygenated. After an equilibration period of 30 min, pancreatic juice was continuously collected in 15-min samples throughout the entire period of the experiment.

**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 gastrin-releasing peptide (GRP; Peninsula) at a concentration of 100 pM for 60 min. GABA (Sigma) at a concentration of 3, 10, 30, or 100 pM 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 pH of the perfusate.

**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 pM was added to the perfusate from 45 min before EFS until the end of the experiment.

**Effects of GABA receptor antagonists, a neuroblocker, and a somatostatin antagonist on GABA action in CCK-stimulated pancreatic exocrine secretion.** GABA receptors were blocked by bicuculline (Tocris, Baldwin, MO), a GABA$_A$ receptor antagonist (14), or saclofen (Tocris), a GABA$_B$ receptor antagonist (2), at a concentration of 10 $\mu$M. Intrapancreatic neuronal activity was inhibited by tetrodotoxin (Sigma) at a concentration of 1 $\mu$M. Action of endogenous somatostatin was eliminated by cyclo-(7-aminoheptanonyl-Phe-$\delta$-Trp-Lys-Thr[BZL]) (Sigma), a somatostatin antagonist (6, 24), at a concentration of 10 $\mu$M. Chemicals were added to the perfusate from 45 min before CCK infusion until the end of the experiment.

**Measurements of pancreatic exocrine secretion.** The volume flow of pancreatic juice was determined by measuring the length of microtube, which had a capacity of 3.8 ml/cm, filled by pancreatic juice in 15 min. The activity of $\alpha$-amylase in pancreatic juice was determined according to a method reported previously (28).

**Statistical analysis of data.** All results are expressed as means $\pm$ SE. The data were analyzed using Student’s t-test. The difference was considered significant when the $P$ value was $<0.05$.

**RESULTS**

**Effects of GABA on secretagogue-induced pancreatic exocrine secretion.** As shown in Fig. 1, the isolated, perfused rat pancreas spontaneously secreted a minute amount of juice (3.70 ± 0.29 $\mu$l/60 min) and amylase activity (46.63 ± 4.15 U/60 min). GABA, given intra-arterially at concentrations of 3, 10, 30, and 100 pM, did not change the spontaneous pancreatic secretions of fluid and amylase. Secretin (12 pM) significantly increased pancreatic secretions of fluid and amylase from the basal levels to 15.63 ± 1.12 $\mu$l/60 min ($P < 0.001$) and 77.65 ± 7.19 U/60 min ($P < 0.05$), respectively. GABA, at all concentrations used in this study, failed to modify the secretin-induced pancreatic exocrine secretion. CCK-8 (10 pM) significantly increased the basal pancreatic secretions of fluid and amylase to 24.59 ± 0.96 $\mu$l/60 min ($P < 0.001$) and 359.39 ± 29.27 U/60 min ($P < 0.001$), respectively, which were further dose-dependently elevated by GABA. GABA, at a concentration of 30 $\mu$M, maximally enhanced the CCK-induced pancreatic secretions of fluid and amylase to levels of 37.39 ± 2.34 $\mu$l/60 min ($P < 0.001$) and 792.42 ± 102.35 U/60 min ($P < 0.001$), respectively. GRP (100 pM) significantly increased the basal pancreatic secretions of fluid and amylase to 17.95 ± 1.47 $\mu$l/60 min ($P < 0.001$) and 265.70 ± 31.35 U/60 min ($P < 0.001$), respectively, which were also further dose-dependently elevated by GABA. GABA, at a concentration of 10 $\mu$M, maximally enhanced the GRP-induced pancreatic secretions of fluid and amylase to levels of 38.91 ± 4.83 $\mu$l/60 min ($P < 0.01$) and 764.64 ± 93.49 U/60 min ($P < 0.01$), respectively.

**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 $\mu$l/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 $\mu$M, further dose-dependently elevated the EFS-induced pancreatic exocrine secretion.

**Fig. 1. Effects of GABA on spontaneous or secretagogue-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 CCK (10 $\mu$M)- or gastrin-releasing peptide (GRP; 100 pM)-induced pancreatic secretions of fluid and amylase but failed to change spontaneous or secretin (12 pM)-induced pancreatic 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 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 (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-α-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

![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.](http://ajpgi.physiology.org/)

![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 μM)-induced pancreatic secretion, but saclofen (10 μM) failed to change it. ND, no significant difference.](http://ajpgi.physiology.org/)

![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 μM)-induced pancreatic secretion even in the presence of TTX (1 μM). The incremental rates are identical to those obtained without TTX.](http://ajpgi.physiology.org/)

![Fig. 5. Effects of somatostatin antagonist on GABA action in CCK-induced pancreatic exocrine secretion.](http://ajpgi.physiology.org/)
were less than one-half of those obtained without the somatostatin antagonist.

**DISCUSSION**

GABA does not appear to have any influence on spontaneous exocrine secretion in the rat pancreas, because GABA at concentrations of 3, 10, 30, and 100 μM did not change spontaneous pancreatic secretion of fluid and amylase in this study. Thus effects of GABA on pancreatic secretion induced by secretagogues were investigated. GABA did not modify pancreatic secretions of fluid and amylase induced by secretin but did increase dose-dependently the secretions induced by CCK. GABA exerted a maximal effect at 30 μM, with elevation of the CCK-induced pancreatic secretions of fluid and amylase by 52.1% and 120.5%, respectively. Because the main function of secretin is stimulation of water and bicarbonate secretions (5) whereas that of CCK is stimulation of enzyme secretion (17), the results of this study suggest that GABA may enhance pancreatic secretion induced by secretagogues that predominantly stimulate enzyme secretion. To test this suggestion, an effect of GABA on pancreatic secretion evoked by GRP, another peptide predominantly stimulating pancreatic enzyme secretion (7, 16), was investigated in this study. GABA also dose-dependently increased pancreatic secretions of fluid and amylase by 117.5% and 202.9%, respectively.

We showed previously (22) that cholinergic neurons and GRPergic neurons in the pancreas are the main 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 evoked by EFS, which was applied to the isolated pancreas to excite intrapancreatic neurons (23, 24). This result suggests that GABA may increase the EFS-induced pancreatic secretion by enhancing the actions of acetylcholine and GRP, which are released during excitation of neurons in the pancreas. The enhancements by GABA of GRP- and EFS-induced pancreatic exocrine secretion provide strong evidence that GABA enhances pancreatic exocrine secretion induced by secretagogues that predominantly stimulate enzyme secretion. Cellular mechanisms of the interaction between GABA and the secretagogues should be elucidated in future studies.

The effects of GABA on CCK-induced pancreatic secretions of fluid and amylase were effectively inhibited by bicuculline, a GABA_A receptor antagonist (14), but not affected by saclofen, a GABA_B receptor antagonist (2), in this study. The results are in good agreement with previous reports that GABA_A receptors exist in pancreatic exocrine cells of neonatal rats (27) and AR42J cells, a pancreatic cancer cell line (3). 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 secretion 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-
nous somatostatin. When the action of endogenous somatostatin was blocked by cyclo-(7-aminoheptanoyl-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 somatostatin 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 predominately stimulate enzyme secretion, via the GABA_A receptors in the rat pancreas. The enhancing effect of GABA is partially mediated by inhibition of islet somatostatin 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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REFERENCES


GABA EFFECT ON PANCREATIC EXOCRINE SECRETION

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