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Effects of γ-Aminobutyric Acid on Intrinsic Cholinergic Action in Exocrine Secretion of Isolated, Perfused Rat Pancreas

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γ-Aminobutyric acid (GABA) has been reported to enhance exocrine secretion evoked not only by secretagogues but also by intrinsic neuronal excitation in the pancreas. The pancreas contains cholinergic neurons abundantly that exert a stimulatory role in exocrine secretion. This study was undertaken to examine effects of GABA on an action of cholinergic neurons in exocrine secretion of the pancreas. Intrinsic neurons were excited by electrical field stimulation (EFS; 15 V, 2 msec, 8 Hz, 45 min) in the isolated, perfused rat pancreas. Tetrodotoxin or atropine was used to block neuronal or cholinergic action. Acetylcholine was infused to mimic cholinergic excitation. GABA (30 μM) and muscimol (10 μM), given intra-arterially, did not change spontaneous secretion but enhanced cholecystokinin (CCK; 10 pM)-induced secretions of fluid and amylase. GABA (3, 10, 30 μM) further elevated EFS-evoked secretions of fluid and amylase dose-dependently. GABA (10, 30, 100 μM) also further increased acetylcholine (5 μM)-induced secretions of fluid and amylase in a dose-dependent manner. Bicuculline (10 μM) effectively blocked the enhancing effects of GABA (30 μM) on the pancreatic secretions evoked by either EFS or CCK. Both atropine (2 μM) and tetrodotoxin (1 μM) markedly reduced the GABA (10 μM)-enhanced EFS- or CCK-induced pancreatic secretions. The results indicate that GABA enhances intrinsic cholinergic neuronal action on exocrine secretion via the $GABA_A$ receptors in the rat pancreas.

Key Words: Acetylcholine, Cholecystokinin, Pancreatic intrinsic neuron, GABA receptor, Pancreatic exocrine secretion

INTRODUCTION

γ-Aminobutyric acid (GABA), a well-known inhibitory neurotransmitter, is abundantly present in islet β-cells of the pancreas at a high concentration comparable to that in the brain (Okada et al, 1976; Garry et al, 1986; Michalik et al, 1993). The pancreatic islets are functionally connected with exocrine cells through the intra-pancreatic circulation, the islet-acinar portal system (Henderson & Daniel, 1979; Lifson et al, 1985). Although it is unknown at the present time whether GABA is released from the islet β-cells into the circulation, there are good evidences suggesting the release of GABA (Gaskins et al, 1995; Smismans et al, 1997). Because high affinity binding sites of GABA have been observed in pancreatic exocrine cells (Reusens-Billen et al, 1984), it is assumed that GABA might play a role in pancreatic exocrine secretion through the islet-acinar portal system if GABA would be secreted from the islet β-cells. However, it remains completely unknown at present whether endogenous GABA influences exocrine secretion in the pancreas.

We recently reported that GABA, given exogenously, elevated exocrine secretion evoked by secretagogues, such as cholecystokinin (CCK) and gastrin-releasing peptide (GRP), which stimulated predominantly enzyme secretion, in the isolated, perfused rat pancreas (Park & Park, 2000; Park et al, 2002). We also observed earlier that GABA increased pancreatic exocrine secretion evoked by electrical field stimulation (EFS) (Park & Park, 2000). Because the EFS-evoked pancreatic secretion was completely inhibited by tetrodotoxin (Park et al, 1998), it was quite likely that GABA might have enhanced exocrine secretion which was evoked by excitation of the intrinsic neurons in the pancreas. Very recently, we reported that the enhancing effect of GABA on the EFS-evoked exocrine secretion was reduced by an anti-GRP antiserum (Park et al, 2002) in the isolated rat pancreas. Since the pancreas contains GRPergic neurons (Moghimzadeh et al, 1983; Knuhtsen et al, 1985; De Giorgio et al, 1992), the result indicates that GABA enhances the action of intrinsic GRPergic neurons on pancreatic exocrine secretion. It is well known that the pancreas also abundantly contains cholinergic neurons (Lakomy & Chodkowska, 1984). Suppression of the cholinergic tone by vagotomy or by infusion of atropine results in reduction of pancreatic exocrine responses to gut

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ABBREVIATIONS: GABA, γ-aminobutyric acid; EFS, electrical field stimulation; CCK, cholecystokinin; GRP, gastrin releasing peptide.

hormones (Debas et al, 1975; Soudah et al, 1992; Li & Owyang, 1993; Park et al, 1998). On the other hand, elevation of the cholinergic tone by electrical stimulation of the vagus nerves or intra-pancreatic nerves, or by infusion of cholinergic agonists results in an increase in the pancreatic responses to gut hormones (Matsumoto & Kano, 1984; Kim et al, 1989; Nelson et al, 1993; Park et al, 1998; Park et al, 1999). Therefore, the cholinergic tone is essential for hormonal action in pancreatic exocrine secretion. However, the effect of GABA on the cholinergic action has not yet been studied at present.

Therefore, the present study was undertaken to investigate whether exogenous GABA could enhance an action of intrinsic cholinergic neurons on exocrine secretion in the rat pancreas. The isolated, perfused pancreas model was used in this study to eliminate possible influences of extrinsic nerves and hormones on pancreatic exocrine secretion.

METHODS

Experimental animals

Male Sprague-Dawley rats, weighing 250~300 g, were anesthetized with an intraperitoneal injection of 25% urethan (Sigma, St. Louis, MO, U.S.A.) at a single dose of 0.7 ml/100 g of body weight after a 24 hour-fast with free access to water. The rats were sacrificed by an intra-venous overdose injection of urethan after isolation of the pancreas.

Preparation of totally isolated, vascularly perfused rat pancreas

The totally isolated, vascularly perfused rat pancreas was prepared according to a method described previously (Park et al, 2000; Park et al, 2000). In brief, the abdominal aorta was carefully dissected and cannulated with PE-50 tubing (Clay Adams, Parsippany, NJ, U.S.A.) just above the celiac axis, and then ligated just below the superior mesenteric artery. The hepatic portal vein was also cannulated with Tygon microbore tubing (Fisher Scientific, Pittsburgh, PA, U.S.A.). The pancreatic duct was cannulated at its duodenal end with PE-10 tubing (Clay Adams) to collect pancreatic secretion. 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. The isolated pancreas was perfused with modified Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg water) through the celiac axis and the superior mesenteric artery at a flow rate of 1.2 ml/min by using a multistaltic pump (Buchler, Kansas, MO, U.S.A.). The perfusate was drained through the hepatic portal vein. The experimental chamber was also continuously supplied with the modified Krebs-Henseleit solution at a flow rate of 0.35 ml/min and oxygenated with 95% O_2-5% CO_2. The modified Krebs-Henseleit solution contained 0.1% bovine serum albumin (Sigma), 3% Dextran T-70 (Sigma) and 18-mM glucose (Sigma) and was continuously oxygenated with 95% O_2-5% CO_2. After an equilibration period of 30 minutes, pancreatic juice was continuously collected in 15-min samples throughout the entire experimental period.

Effects of cholinergic blocking on GABA-enhanced cholecystokinin-induced pancreatic exocrine secretion

Pancreatic exocrine secretion was stimulated by intra-arterial infusion of 10 pM cholecystokinin (CCK)-8 (Squibb Institute, Princeton, NJ, U.S.A.) for 60 min. GABA (Sigma; 30 μM) or muscimol (Sigma; 10 μM), a $GABA_A$ receptor agonist, was added in the perfusate from 60 minutes before the CCK infusion until the end of the experiment. To antagonize the actions of GABA agonists, 10 pM bicuculline (Tocris, Baldwin, MO, U.S.A.), a $GABA_A$ receptor antagonist (Gilon et al, 1991; Park et al, 2000), was also infused to the pancreas from 60 minutes before the CCK infusion until the end of the experiment. For inhibition of the basal tone of all neurons or cholinergic neurons, tetrodotoxin (Sigma; 1 μM) or atropine (Sigma; 2 μM) was intra-arterially administered to the isolated pancreas from 60 minutes before the CCK infusion until the end of the experiment.

Effects of cholinergic blocking on GABA-enhanced electrical field stimulation (EFS)-evoked pancreatic exocrine secretion

Neurons in the isolated, perfused rat pancreas were excited by application of EFS (15 V, 2 msec, 8 Hz for 45 min) according to the method described previously (Park et al, 1998; Park et al, 2000). Effects of GABA on the EFS-evoked pancreatic exocrine secretion were determined by intra-arterial infusion of GABA, at a concentration of 3, 10 or 30 μM, to the isolated pancreas from 60 minutes before EFS began until the end of the experiment. GABA, at all concentrations used in this experiment, did not change the pH of the perfusate. Bicuculline (10 μM) was also infused to antagonize the GABA (10 μM) action from 60 minutes before EFS began until the end of the experiment. To prevent activation of all the intrinsic neurons or cholinergic neurons selectively, tetrodotoxin (1 μM) or atropine (2 μM) was intra-arterially administered to the isolated pancreas from 60 minutes before EFS began until the end of the experiment.

Effects of GABA on acetylcholine-induced pancreatic exocrine secretion

To mimic cholinergic excitation, 5 μM acetylcholine (Sigma) was intra-arterially infused to the isolated rat pancreas for 60 minutes. GABA at a concentration of 10, 30 or 100 μM was added to the perfusate from 60 minutes before the acetylcholine infusion until the end of the experiment.

Measurements

The fluid secretion of the isolated pancreas was determined by measuring the length of microtube, which had a capacity of 3.8 μl/cm, filled by pancreatic juice for 15 minutes (Park et al, 2000). The activity of α-amylase in pancreatic juice was measured according to the method described elswhere (Rick & Stegbauer, 1974).

Statistical analysis

All data are presented as mean±SE. The data were analyzed using the Student's t-test. The difference was

Table 1. Effects of bicuculline on GABA- and muscimol-enhanced cholecystokinin (CCK)-evoked secretions of fluid and amylase in the isolated, perfused rat pancreas

	Cholecystokinin					
	Basal	Control	GABA		Muscimol	
			−Bicuculline	+Bicuculline	−Bicuculline	+Bicuculline
Fluid (L/60 min)	3.22±0.55	17.28±1.29	25.12[a]±1.91	17.41[b]±1.66	22.11[a]±2.33	16.46[b]±1.87
Amylase (U/60 min)	30.39±9.38	295.65±30.59	798.38[a]±115.07	467.90[b]±48.27	538.02[a]±91.36	378.74[b]±37,75

Each value represents mean±SE of 6 pancreata. [a]: significant difference ($P<0.05$) compared with the corresponding value of CCK alone (control). [b]: significant difference ($P<0.01$) compared with the corresponding value obtained without bicuculline. GABA (30 μM) and muscimol (10 μM) further elevated the CCK (10 pM)- evoked pancreatic secretions of fluid and amylase, which was markedly reduced by bicuculline (10 μM).

considered significant when the P value was less than 0.05.

RESULTS

Effects of GABA agonists on CCK-induced pancreatic exocrine secretion

As shown in Table 1, the isolated, perfused rat pancreas spontaneously secreted fluid and amylase at rates of 3.22±0.55 μl/60 min and 30.39±9.38 U/60 min, respectively. CCK-8 at 10 pM remarkably increased the spontaneous secretions of fluid and amylase to 17.28±1.29 μl/60 min and 295.65±30.59 U/60 min, respectively. GABA at 30 μM further elevated the CCK-induced secretions of fluid and amylase by 45.4% and 170.0%, respectively. Muscimol (10 μM), a GABA agonist, also further elevated the CCK-induced secretions of fluid and amylase by 28.0% and 82.0%, respectively. Bicuculline (10 μM), a $GABA_A$ receptor antagonist, strikingly reduced the enhancing effects of not only GABA (inhibitions of fluid and amylase secretions by 30.7% and 41.4%, respectively) but also muscimol (inhibitions of fluid and amylase secretions by 25.6% and 19.1%, respectively) on the CCK-induced pancreatic secretions.

Effects of cholinergic blocking on GABA-enhanced CCK-induced pancreatic exocrine secretion

Effects of neuroblockers on the GABA action in CCK-induced pancreatic secretions of fluid and amylase are illustrated in Fig. 1. Atropine (2 μM) significantly reduced the CCK-induced secretions of fluid and amylase to 10.50±1.22 μl/60 min ($P<0.001$) and 185.56±19.63 U/60 min ($P<0.001$), respectively and the GABA-enhanced CCK-induced secretions of fluid and amylase to 21.22±2.30 μl/60 min ($P<0.05$) and 389.52±43.63 U/60 min ($P<0.001$), respectively. Tetrodotoxin (1 μM) also significantly diminished the CCK-induced secretions of fluid and amylase to 8.12±2.23 μl/60 min ($P<0.001$) and 142.34±20.45 U/60 min ($P<0.001$), respectively and the GABA-enhanced CCK-induced secretions of fluid and amylase to 16.64±2.52 μl/60 min ($P<0.001$) and 284.36±42.52 U/60 min ($P<0.001$), respectively. However, GABA still further elevated the CCK-induced secretions even if atropine (increases of fluid and amylase secretions by 102.1% and 110.0%, respectively) or tetrodotoxin (increases of fluid and amylase secretions by 100.0% and 97.5%, respectively) was present.

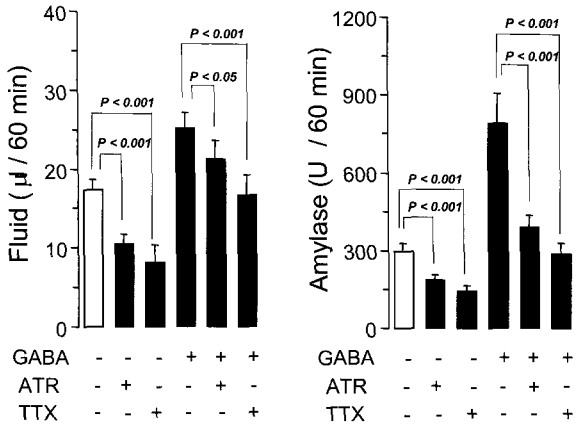

Fig. 1. Effects of atropine (ATR) and tetrodotoxin (TTX) on the GABA-enhanced CCK-evoked secretions of fluid (*Left*) and amylase (*Right*) in the isolated, perfused rat pancreas. Each value represents mean±SE of 6 pancreata. GABA (30 μM) further increased the CCK-evoked pancreatic secretions. The enhancing effects of GABA are significantly ($P<0.05$) reduced by atropine (2 μM) and tetrodotoxin (1 μM). However, GABA still further increased the CCK-induced pancreatic secretions even in the presence of atropine or tetrodotoxin.

Effects of GABA on EFS-evoked pancreatic exocrine secretion

Fig. 2 shows dose-dependent effects of GABA on EFS-evoked pancreatic secretions of fluid and amylase. EFS, at an intensity of 15 V, 2 msec, 8 Hz and 45 min, significantly increased secretions of fluid ($P<0.05$) and amylase ($P<0.001$) from the basal levels to 13.17±1.04 μl/45 min and 196.78±16.88 U/45 min, respectively. GABA at concentrations of 3, 10 and 30 μM further dose-dependently elevated the EFS-evoked secretions of fluid and amylase. As shown in Fig. 3, bicuculline (10 μM) completely abolished the enhancing effects of GABA (10 μM) on the EFS-evoked pancreatic.

Effects of cholinergic blocking on GABA-enhanced EFS-evoked pancreatic exocrine secretion

Fig. 4 shows effects of neuroblockers on the GABA action

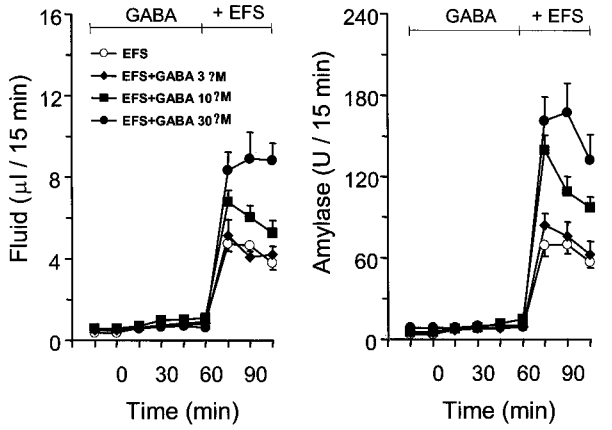

Fig. 2. Dose-dependent effects of GABA on electrical field stimulation (EFS)-evoked secretions of fluid (*Left*) and amylase (*Right*) in the isolated, perfused rat pancreas. Each value represents mean±SE of 6 pancreata. GABA (3, 10, 30 μM) further elevated the EFS (15 V, 2 msec, 8 Hz, 45 min)-evoked pancreatic secretions of fluid and amylase dose-dependently.

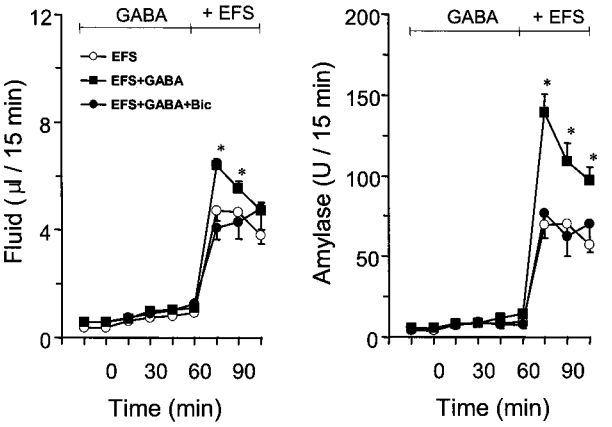

Fig. 3. Effects of bicuculline (Bic) on the GABA-enhanced EFS-evoked secretions of fluid (*Left*) and amylase (*Right*) in the isolated, perfused rat pancreas. Each value represents mean±SE of 6 pancreata. *: significant difference ($P<0.05$) compared with the corresponding value of EFS alone. Bicuculline (10 μM) completely eliminated the enhancing effects of GABA (10 μM) on the EFS (15 V, 8 Hz, 2 msec, 45 min)-evoked pancreatic secretions of fluid and amylase.

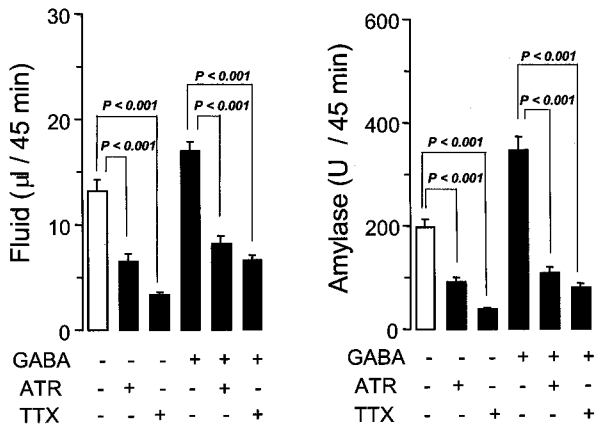

Fig. 4. Effects of atropine (ATR) and tetrodotoxin (TTX) on the GABA-enhanced EFS-evoked evoked secretions of fluid (*Left*) and amylase (*Right*) in the isolated, perfused rat pancreas. Each value represents mean±SE of 6 pancreata. GABA (10 μM) further increased the EFS (15 V, 8 Hz, 2 msec, 45 min)-evoked pancreatic secretions. The enhancing effects of GABA on the EFS-evoked secretions were significantly reduced ($P<0.001$) by atropine (2 μM) or tetrodotoxin (1 μM). However, GABA still further increased the EFS-evoked pancreatic secretions even in the presence of atropine or tetrodotoxin.

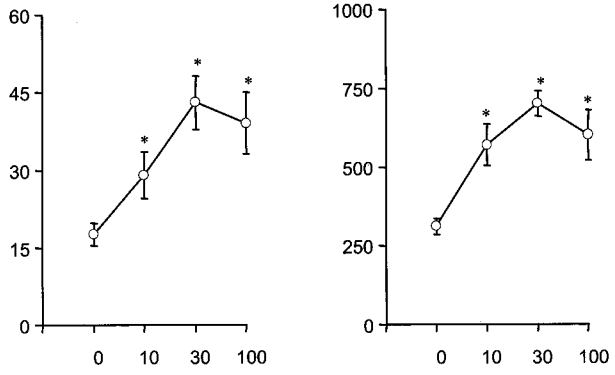

Fig. 5. Dose-dependent effects of GABA on acetylcholine-evoked secretions of fluid (*Left*) and amylase (*Right*) in the isolated, perfused rat pancreas. Each value represents mean±SE of 6 pancreata: significant difference ($P<0.05$) compared with the corresponding value obtained without GABA. GABA (10, 30, 100 μM) further elevated the acetylcholine (5 μM)-evoked pancreatic secretions of fluid and amylase dose-dependently.

in EFS-evoked pancreatic secretions of fluid and amylase. Atropine (2 μM) significantly reduced the EFS-evoked secretions of fluid and amylase to 6.47±0.77 μl/45 min ($P<$ 0.001) and 92.13±7.96 U/45 min ($P<0.001$), respectively, and the GABA (10 μM)-enhanced EFS-evoked secretions of fluid and amylase to 8.16±0.78 μl/45 min ($P<0.001$) and 108.07±11.40 U/45 min ($P<0.001$), respectively. Tetrodotoxin (1 μM) also significantly diminished the EFS-evoked secretions of fluid and amylase to 3.33±0.22 μl/45 min ($P<$ 0.001) and 37.08±4.41 U/45 min ($P<0.001$), respectively and the GABA (10 μM)-enhanced EFS-evoked secretions of fluid and amylase to 6.56±0.52 μl/45 min ($P<0.001$) and 78.61±9.88 U/45 min ($P<0.001$), respectively. However, GABA (10 μM) still further elevated the EFS-evoked secretions even if atropine (increases of fluid and amylase secretions by 26.1% and 17.3%, respectively) or tetrodotoxin (increases of fluid and amylase secretions by 97.0% and 112.0%, respectively) was present.

Effects of GABA on acetylcholine-induced pancreatic exocrine secretion

Fig. 5 demonstrates dose-dependent effects of GABA on acetylcholine-induced pancreatic exocrine secretion. Acetylcholine at 5 μM remarkably increased secretions of fluid and amylase from the basal levels to 14.39±1.57 μl/60 min ($P<0.001$) and 282.15±16.23 U/60 min ($P<0.001$), respec-

tively. GABA at concentrations of 10, 30 and 100 μM further dose-dependently elevated the acetylcholine-induced pancreatic secretions.

DISCUSSION

In the present study, GABA further elevated the CCK-induced secretions of fluid and amylase in the totally isolated perfused rat pancreas, which confirms our earlier work (Park et al, 2000). Because GABA can be used as an energy source, a synthetic GABA agonist as well as a GABA antagonist was used to confirm the effects of GABA effects in the present study. Muscimol, a $GABA_A$ receptor agonist, also enhanced the CCK-induced pancreatic secretions. The enhancing effects of both GABA and muscimol were markedly reduced by bicuculline, a $GABA_A$ receptor antagonist (Gilon et al, 1991; Park et al, 2000). Therefore, it is highly likely that GABA exerted the enhancing effects by acting on the receptor rather than by being metabolized to produce energy. GABA appeared to affect the amylase secretion much stronger (~threefold) than the fluid secretion when the secretions were stimulated by CCK. In this study, the incremental rates by GABA agonists of the CCK-induced amylase secretion (170.0% increase by GABA and 82.0% increase by muscimol) were much higher than the rates of the fluid secretion (45.4% increase by GABA and 28.0% increase by muscimol). However, GABA alone did not seem to affect pancreatic exocrine function because both GABA and muscimol did not change spontaneous pancreatic secretion in this study. GABA also did not modify pancreatic secretions induced by secretin, which predominantly stimulates water and bicarbonate secretion (Park et al, 2000).

In consistence with our previous result (Park et al, 2000), the GABA action on CCK-induced pancreatic secretions was partially reduced by tetrodotoxin and atropine. However, GABA further increased the CCK-induced secretions of fluid and amylase even if tetrodotoxin or atropine was present. The results suggest that GABA is able to effectively elevate the CCK-induced pancreatic secretions when the basal activity of the intrapancreatic cholinergic neuron is preserved. However, it also raises a question that GABA might modify cholinergic action on pancreatic secretions. In answer to the question, we firstly observed effects of GABA on pancreatic secretions evoked by intrinsic neuronal activation. GABA further dose-dependently elevated the EFS-evoked pancreatic secretions of fluid and amylase. The GABA-enhanced EFS-evoked secretions were markedly diminished by tetrodotoxin. Because tetrodotoxin inhibits the pancreatic secretions evoked by EFS alone as shown in this study as well as others (Park et al, 1998), which suggests activation of intrinsic neurons by EFS to stimulate the secretions, it is likely that GABA elevates pancreatic secretions induced by intrinsic neuronal activation. Secondly, we investigated effects of GABA on cholinergic action in pancreatic secretions. The cholinergic neurons, most abundantly present in the pancreas (Lakomy & Chdkowska, 1984), appear to be excited by EFS because atropine, a well-known cholinergic receptor antagonist, reduces pancreatic secretions evoked by EFS alone as shown in this study as well as others (Park et al, 1999). When cholinergic transmission in the isolated pancreas was blocked with atropine, the effects of GABA on the EFS-evoked pancreatic secretions of fluid and amylase were markedly reduced. Thus, acetylcholine, a cholinergic neurotransmitter, was infused to the pancreas to mimic cholinergic activation: Acetylcholine increased pancreatic secretions of fluid and amylase, and the pancreatic responses to acetylcholine were further dose-dependently elevated by GABA. The results described herein provide strong evidences that GABA enhances the cholinergic action on pancreatic exocrine secretion by elevating the action of acetylcholine, which is released during cholinergic excitation, although release of acetylcholine by EFS was not determined.

GABA also seems to enhance pancreatic exocrine secretion evoked by non-cholinergic intrinsic neurons because the GABA-enhanced EFS-evoked pancreatic secretions were incompletely inhibited by atropine (51.7% inhibition of fluid and 68.7% inhibition of amylase). The pancreas includes neurons containing GRP (Moghimzadeh et al, 1983; Knuhtsen et al, 1985; De Georgio et al, 1992), vasoactive intestinal polypeptide (VIP; Holst et al, 1984) and other peptides (Karen et al, 1997). We have previously reported that an anti-GRP antiserum reduced the GABA-enhanced EFS-evoked pancreatic secretion, suggesting that GABA may enhance the GRPergic action on pancreatic secretion (Park et al, 2002). It appears to be worthy to elucidate in the future whether GABA also enhances the action of other peptidergic neurons, including VIP.

GABA does not seem to change the intrinsic cholinergic activity to alter pancreatic exocrine secretion because GABA and muscimol did not change spontaneous pancreatic secretion but still elevated CCK-induced pancreatic secretion even after cholinergic transmission was inhibited by atropine. It has been demonstrated that GABA-containing neuronal cell bodies locate at the periphery of islets and that numerous GABA-containing processes of the cells extend to the exocrine pancreas (Sorenson et al, 1991). Very recently, GABA sensitive neurons were observed in the cat pancreas (Sha et al, 2001). Although GABA depolarized all ganglial cells recorded in the study through $GABA_A$ receptors, only ~10% neurons generated action potential. It is not clear at the present time whether the GABA-containing neurons or the GABA sensitive neurons affect pancreatic exocrine secretion.

In this study, bicuculline, a $GABA_A$ receptor antagonist, was also found to inhibit the effects of GABA on pancreatic secretions of fluid and amylase evoked by CCK and EFS. The $GABA_A$ receptor has been recognized in pancreatic exocrine cells of the neonatal rat (Reusens-Billen et al, 1984) and in AR42J cells, a pancreatic cancer cell line (von Blankenfeld et al, 1995). Thus, GABA seems to exert the enhancing effect on the cholinergic neuronal action via $GABA_A$ receptors in the rat pancreas. $GABA_B$ receptors do not seem to mediate the GABA effect on pancreatic exocrine secretion because saclofen, a $GABA_B$ receptor antagonist, did not modify the GABA effect on CCK-induced pancreatic secretions (Park et a, 2000). A cellular mechanism of the GABA action on pancreatic acinar cells remains completely unknown at present. The $GABA_A$ receptor appears to mediate membrane potential changes depending on cell types. It has been reported that GABA activates $GABA_A$ receptor-mediated chloride channels in glucagon cells, resulting in hyperpolarization and reduction of glucagon secretion (Rorsman et al, 1989). On the contrary, however, $GABA_A$ receptors mediate depolarization of AH/type II and S/type I myenteric neurons with chloride-dependent, bicuculline-sensitive process (Cherubini & North, 1984).

The cellular mechanism of the GABA action on pancreatic exocrine secretion also remains to be elucidated in future studies.

In summary, GABA also dose-dependently increased the pancreatic secretions evoked by EFS and further elevated CCK-induced secretions of fluid and amylase in the isolated perfused rat pancreas. Tetrodotoxin as well as atropine reduced the GABA-enhanced CCK- or EFS-evoked pancreatic secretions. GABA dose-dependently elevated pancreatic exocrine secretion induced by exogenous acetylcholine. Bicuculline inhibited the GABA effect on the CCK- or EFS-evoked pancreatic secretions. Thus, these results lead us to conclude that GABA enhances intrinsic cholinergic action on exocrine secretion through $GABA_A$ receptors in the rat pancreas.

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