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Effects of γ-Aminobutyric Acid on Pancreatic Amylase Secretion Evoked by Sodium Oleate in Anesthetized Rats

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γ-Aminobutyric Acid (GABA) is contained in pancreatic islet β-cells although its physiological role in pancreatic exocrine function is completely unknown at the present time. Recently, we have reported that exogenous GABA enhances secretagogue-evoked exocrine secretion in the isolated, perfused rat pancreas. This study was aimed to investigate an effect of exogenous GABA on pancreatic exocrine secretion *in vivo* evoked by intestinal stimulation. Rats were anesthetized with urethane (1.4 g/kg) after 24-h fast with free access to water. GABA (10, 30 and 100 μmol/kg/h), given intravenously, did not change spontaneous pancreatic amylase secretion but dose-dependently elevated the amylase secretion evoked by intraduodenal sodium oleate (0.05 *m*mol/h). GABA (30 μmol/kg/h) also further increased the amylase secretion stimulated by CCK (30 *p*mol/kg/h) plus secretin (20 *p*mol/kg/h) but failed to modify the amylase secretion induced by secretin alone. GABA (10, 30 and 100 μmol/kg/h) also dose-dependently elevated pancreatic amylase secretion evoked by CCK alone. Bicuculline (100 μmol/kg/h), a $GABA_A$-receptor antagonist, markedly reduced the GABA-enhanced pancreatic responses to sodium oleate, CCK plus secretin or CCK alone. The results indicate that GABA enhances the sodium oleate-evoked pancreatic amylase secretion via $GABA_A$-receptors in anesthetized rats, which may account for elevating the action of CCK released by sodium oleate.

Key Words: GABA, Sodium oleate, Cholecystokinin, Secretin, Pancreatic exocrine secretion, $GABA_A$-receptor

INTRODUCTION

β-Cells in the Langerhans' islet of the pancreas contain γ-aminobutyric acid (GABA) at a high concentration comparable to that in the brain (Okada et al, 1976; Garry et al, 1986; Michalik et al, 1993). Existence of glutamate decarboxylase and GABA transaminase in β-cells (Vincent et al, 1983; Sakaue et al, 1987) indicates that GABA is synthesized and catabolized in the cells. Although release of GABA from β-cells has not been demonstrated in the pancreas, there are good evidences that GABA may be released from the cells. β-cells contain synaptic-like microvesicles that may be concerned with GABA secretion (Reetz et al, 1991). The pancreatic β-TC6 cells, a murine β-cell line developed from insulinoma, secrete GABA by high concentration of glucose (Gaskins et al, 1995). Furthermore, cultured rat β-cells secrete GABA in response to glutamine dose-dependently (Smismans et al, 1997). Thus, it is assumed that GABA, if it is released from the β-cells, may reach to pancreatic acinar cells through the islet-acinar portal system (Henderson & Daniel, 1979; Lifson et al, 1985). However, an effect of endogenous GABA on pancreatic exocrine function is completely unknown at the present time although high-affinity binding sites of GABA have been determined in the exocrine pancreas (Reusens-Billen et al, 1984).

Very recently, we have firstly reported that exogenous GABA modifies pancreatic exocrine secretion stimulated by secretagogues in the totally isolated, perfused rat pancreas (Park & Park, 2000). In the isolated pancreas, GABA further elevated cholecystokinin (CCK)-stimulated pancreatic exocrine secretion but did not change secretin-evoked one. The GABA-enhanced CCK-stimulated pancreatic secretion was abolished by bicuculline, a $GABA_A$-receptor antagonist (Gilon et al, 1991). Thus, it is suggested that GABA may enhance pancreatic exocrine secretion induced by secretagogues, which predominantly stimulates enzyme secretion via $GABA_A$-receptors in the isolated rat pancreas. However, it is unknown whether GABA also exerts the enhancing effect on pancreatic exocrine secretion evoked by physiological stimulation *in vivo*.

Therefore, the present investigation was aimed to see effects of exogenous GABA on pancreatic amylase secretion evoked by an intestinal stimulation in anesthetized rats. Sodium oleate was intraduodenally administered to mimic the intestinal stimulation. Effects of exogenous GABA on pancreatic amylase secretion stimulated by exogenous CCK and secretin were also investigated because sodium oleate in the duodenum increased releases of CCK and secretin

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ABBREVIATIONS: GABA, γ-aminobutytic acid; CCK, cholecystokinin; Sec, secretin.

(Li et al, 1990).

METHODS

Animal preparation for collection of pancreatic juice

Male Sprague-Dawley rats, weighing 250~300 g, were anesthetized with an intraperitoneal injection of 20% urethan (Sigma, St. Louis, MO, U.S.A.) at a dose of 0.7 ml/100 g body wt after 24-h fast with free access to water. A PE-50 tubing (Clay Adams, Parsippany, NJ, U.S.A.) was placed in the external jugular vein and then connected to a syringe- driving pump for infusion of chemicals. The pancreatic duct was cannulated with a PE-10 tubing (Clay Adams) at its duodenal end to collect pancreatic juice. The bile duct was cannulated with a PE-10 tubing at its hepatic end to divert bile juice into the jejunum. The gastroduodenal junction was tightly ligated to prevent passage of gastric juice into the duodenum. Rats were placed on a heating pad to maintain body temperature at 37°C after covering the abdominal wound with a piece of saline-moistened gauze. After a 30-min stabilization period, pancreatic juice was collected in 15-min samples throughout the entire period of the experiment. Rats were sacrificed by an intravenous overdose injection of urethan after the experiment.

Effects of GABA on sodium oleate-stimulated pancreatic exocrine secretion

A polyethylene catheter (PE-50 tubing) was inserted into the proximal end of the duodenum through the stomach to administer sodium oleate solution. Sodium oleate (Sigma) was dissolved in 0.9% NaCl at a concentration of 0.05 mmol/ml. pH of the sodium oleate solution was adjusted to 7.5 by HCl. The sodium oleate solution was administered into the duodenal lumen at a flow rate of 4 ml/h for 90 min after a 30-min basal period of saline infusion. GABA (Sigma) was dissolved in 0.9% NaCl solution, and was intravenously infused at a dose of 10, 30 or 100 μmol/kg/h from 30 min before the sodium oleate administration until

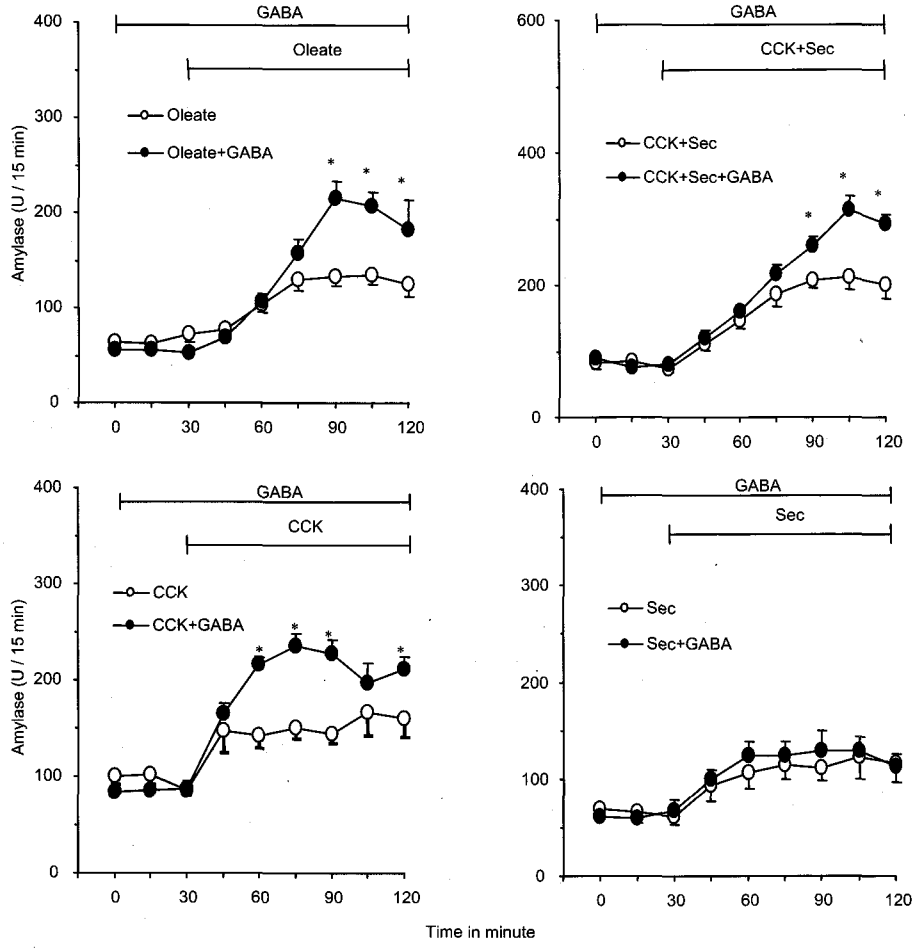

Fig. 1. Effects of γ-aminobutyric acid (GABA) on pancreatic amylase secretion evoked by sodium oleate, cholecystokinin (CCK) plus secretin, CCK alone or secretin alone in anesthetized rats. Each value represents mean±SE of 6 rats. Asterisks indicate the values are significantly different ($P<0.05$) from corresponding value obtained without GABA. GABA (30 μmol/kg/h) further increased pancreatic amylase secretion evoked by sodium oleate (0.05 mmol/h), CCK (30 pmol/kg/h) plus secretin (20 pmol/kg/h) and CCK alone but did not change that evoked by secretin alone.

the end of the experiment. Bicuculline (Tocris Baldwin, MO, U.S.A.), a well-known $GABA_A$-receptor antagonist (Gilon et al, 1991; Park et al, 2000), was intravenously given at a dose of 100 μmol/kg/h from 30 min before the sodium oleate administration until the end of the experiment.

Effects of GABA on CCK-and/or secretin-evoked pancreatic exocrine secretion

Sulfated CCK-8 (Squibb Institute, Princeton, NJ, U.S.A.) and synthetic porcine secretin (Peninsula, Belmont, CA, U.S.A.) were dissolved in 0.9% NaCl solution containing 0.5% bovine serum albumin (Sigma). CCK (30 *p*mol/kg/h) and/or secretin (20 *p*mol/kg/h) were intravenously infused for 90 min after a 30-min basal period of saline infusion. GABA in the presence or absence of bicuculline was intravenously given from 30 min before the peptide infusion until the end of the experiment.

Measurements of pancreatic secretion

The volume flow of pancreatic juice was determined by measuring the length of microtube, which had a capacity of 3.8 μl/cm, filled by pancreatic juice for 15 min. The activity of α-amylase in pancreatic juice was measured by a method described previously (Rick & Stegbauer, 1974; Park et al, 2000).

Statistical analysis

All data were illustrated as means±SE. The data were analyzed using the Student's *t*-test. The difference was considered significant when the P value was <0.05.

RESULTS

Effects of GABA on sodium oleate-stimulated pancreatic exocrine secretion

In the basal period, anesthetized rats spontaneously secreted pancreatic amylase at a rate of 267.76±11.62 U/60 min. Sodium oleate (0.05 *m*mol/h), given into the duodenal lumen, significantly increased ($P<0.01$) pancreatic amylase secretion from the basal level to 499.39±33.79 U/60 min during last 60 minutes of the infusion (Fig. 1). As shown in Fig 2, GABA, given intravenously at a dose of 10, 30 or 100 μmol/kg/h, further elevated the sodium oleate-evoked amylase secretion to 600.02±38.13, 686.98±49.08 or 718.18 ±52.28 U/60 min, respectively. Bicuculline (100 μmol/kg/h) significantly reduced ($P<0.05$) the GABA (30 μmol/kg/h)-enhanced sodium oleate-evoked amylase secretion (Table 1). GABA did not exert any influence on spontaneous pancreatic amylase secretion (data not shown).

Effects of GABA on CCK-and/or secretin-evoked pancreatic exocrine secretion

Effects of GABA (30 μmol/kg/h) on pancreatic amylase secretion evoked by intravenous infusion of CCK (30 *p*mol/kg/h) and/or secretin (20 *p*mol/kg/h) are illustrated in Fig.

Table 1. Effects of bicuculline on the γ-aminobutyric acid (GABA) action in pancreatic amylase secretion (U/60 min) evoked by sodium oleate, cholecystokinin (CCK) plus secretin, CCK alone or secretin alone in anesthetized rats

	Control	GABA	
		−Bicuculline	+Bicuculline
Sodium oleate	499.39±33.79	686.98±48.73	507.28±31.73*
CCK+secretin	756.96±28.90	945.87±24.25	771.22±28.24*
CCK alone	602.21±50.28	874.80±93.70	620.81±24.37*
Secretin alone	455.08±56.04	505.30±45.30	–

Data represent mean±SE of 6 rats. Asterisks indicate that bicuculline significantly reduced ($P<0.05$) the GABA effect.

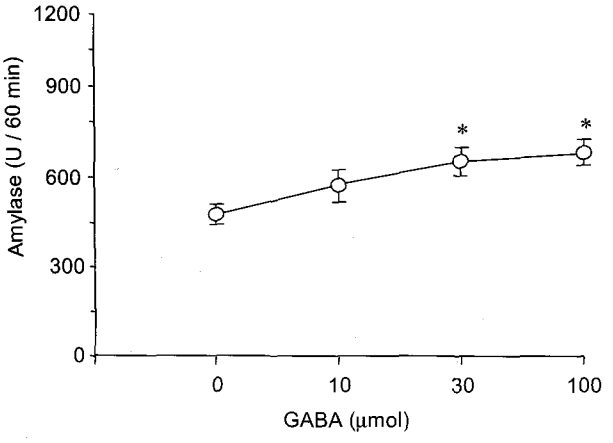

Fig. 2. Dose-dependent effects of γ-aminobutyric acid (GABA) on pancreatic amylase secretion evoked by sodium oleate in anesthetized rats. Each value represents mean±SE of 6 rats. Asterisks indicate the values are significantly different ($P<0.05$) from corresponding value obtained without GABA. GABA enhanced pancreatic amylase secretion evoked by sodium oleate (0.05 *m*mol/h) dose-dependently.

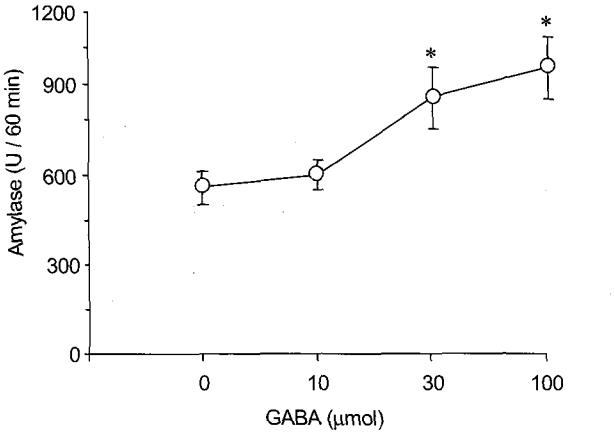

Fig. 3. Dose-dependent effects of γ-aminobutyric acid (GABA) on pancreatic amylase secretion evoked by cholecystokinin (CCK) alone in anesthetized rats. Each value represents mean±SE of 6 rats. Asterisks indicate the values are significantly different ($P<0.05$) from corresponding value obtained without GABA. GABA enhanced pancreatic amylase secretion evoked by CCK (30 *p*mol/kg/h) dose-dependently.

1. Simultaneous infusion of CCK and secretin significantly increased ($P<0.01$) pancreatic amylase secretion from the basal level of 317.98±4.84 to 756.96±28.90 U/60 min during the later 60 minutes of the infusion. GABA further elevated the amylase secretion evoked by CCK plus secretin to 945.87±24.25 U/60 min. Bicuculline (100 μmol/ kg/h) significantly reduced ($P<0.05$) the GABA-enhanced pancreatic response to CCK plus secretin (Table 1). CCK significantly increased ($P<0.01$) pancreatic amylase secretion from the basal level of 373.76±4.84 to 602.21±50.28 U/60 min during last 60 minutes of the infusion. As shown in Fig. 3, GABA, at a dose of 10, 30 or 100 μmol/kg/h, further elevated the CCK-induced amylase secretion to 639.75± 46.82, 874.80±41.55 or 967.30±49.86 U/60 min, respectively. Bicuculline (100 μmol/kg/h) significantly reduced ($P<$ 0.05) the GABA-enhanced CCK-evoked amylase secretion (Table 1). Secretin also significantly increased ($P<0.01$) pancreatic amylase secretion from the basal level of 253.40 ±4.84 to 455.08±56.04 U/60 min during the later 60 minutes of the infusion. However, GABA failed to modify the secretin-induced amylase secretion (Table 1).

DISCUSSION

To mimic the intestinal phase of pancreatic exocrine secretion, sodium oleate was administered into the duodenal lumen of anesthetized rats because sodium oleate, a digestion product of fat, was known to stimulate pancreatic exocrine secretion in rats (Li et al, 1990). In this study, intraduodenal administration of sodium oleate at a rate of 0.2 mmol/h resulted in a marked increase in pancreatic amylase secretion as reported previously (Li et al, 1990). GABA, given intravenously at a dose of 10, 30 or 100 μmol/kg/h, further elevated the pancreatic amylase secretory response to sodium oleate dose-dependently. However, GABA alone did not affect spontaneous pancreatic amylase secretion. The results strongly indicate that GABA does not give any influence on spontaneous pancreatic amylase secretion but enhances the amylase secretion evoked by sodium oleate administered intraduodenally. It is, thus, suggested that GABA may enhance pancreatic exocrine secretion *in vivo*, which is evoked by physiological stimulation.

It has been shown that sodium oleate, administered intraduodenally, induces release of CCK and secretin, which results in stimulation of pancreatic exocrine secretion in anesthetized rats (Li et al, 1990). Thus, effects of GABA on pancreatic amylase secretion stimulated by the two hormones were investigated in anesthetized rats. GABA further increased pancreatic amylase secretion induced by simultaneous infusion of CCK and secretin. GABA did not modify the secretin-evoked amylase secretion but dose-dependently elevated the CCK-evoked amylase secretion. The result is quite similar to our previous observation that GABA does not affect secretin-stimulated exocrine secretion but enhances CCK-evoked exocrine secretion in the isolated, perfused rat pancreas (Park & Park, 2000). Therefore, it is likely that GABA may elevate sodium oleate-stimulated pancreatic amylase secretion by enhancing the action of CCK, which is released by sodium oleate. Although effects of GABA on secretions of CCK and secretin have not been determined in the present study, GABA does not seem to induce release of the gut hormones because GABA did not change spontaneous pancreatic amylase secretion at the basal state. To our knowledge, there is no documentation available at present indicating that GABA affects releases of gut hormones except pancreatic islet hormones. It has been reported that GABA or its agonist inhibits release of somatostatin (Robbins et al, 1981) and glucagon (Rorsman et al, 1989), whereas it stimulates release of insulin (Gerber & Hare, 1980; Robbins et al, 1981).

GABA appears to affect pancreatic amylase secretion via $GABA_A$-receptors because GABA's enhancement of sodium oleate- or CCK-stimulated pancreatic amylase secretion was successfully reduced by bicuculline, a $GABA_A$-receptor antagonist (Gilon et al, 1991; Park & Park, 2000). Very similar results were also observed in our previous experiment (Park & Park, 2000) in which the enhancing effects of GABA on CCK-stimulated fluid and amylase secretions were also inhibited by bicuculline in the isolated rat pancreas. Existence of $GABA_A$ receptors in pancreatic acinar cells of the neonatal rat (Reusens-Billen et al, 1984) and AR42J cells, a pancreatic cancer cell line of the rat (von Blankenfeld et al, 1995) strongly supports our results. It has been reported that the $GABA_A$-receptor mediates depolarization of AH/type II and S/type I myenteric neurons with the chloride-dependent, bicuculline-sensitive process (Cherubini & North, 1984). On the contrary, it has also been reported that activation of $GABA_A$-receptors induces an inward chloride current, which causes inhibition of nerve cell excitation, by hyperpolarization (Schwartz, 1988). GABA also activates $GABA_A$-receptor chloride channels on glucagon cells, which results in hyperpolarization and reduction of glucagon secretion (Rorsman et al, 1989). Thus, a cellular mechanism of the GABA action on pancreatic exocrine secretion remains to be elucidated in future studies. $GABA_B$ receptors do not seem to mediate the GABA effect on pancreatic exocrine secretions because saclofen, a $GABA_B$ receptor antagonist, did not modify the GABA effect on CCK-induced pancreatic exocrine secretion (Park & Park, 2000). A role of the $GABA_C$ receptor in pancreatic exocrine secretion has not been studied yet.

Although effects of exogenous GABA on pancreatic amylase secretion were clearly shown in this study as well as in our previous study (Park & Park, 2000), a physiological role of endogenous GABA in pancreatic exocrine function is still completely unknown. It has been reported that islet hormones give great influences on pancreatic exocrine function via the islet-acinar portal system, which is a portal system formed between the islets and the exocrine pancreas (Henderson & Daniel, 1979; Lifson et al, 1985). Thus, it is assumed that GABA in the β-cells of the pancreatic islets may be released and may influence pancreatic exocrine cells through the islet-acinar portal system. However, there is no confirmed data available on GABA release from the pancreatic islets although GABA is reportedly secreted from the cultured pancreatic β-TC6 cells by high concentration of glucose (Gaskins et al, 1995) and also from the cultured rat β-cells by glutamine (Smismans et al, 1997). In a study (Sorenson et al, 1991), it has been demonstrated that GABA-containing neuronal cell bodies locate at the periphery of islets and numerous GABA-containing processes of the cells extend into the exocrine pancreas. A role of the GABA-containing neurons in pancreatic exocrine function is completely unknown. Nevertheless, endogenous release of GABA in the pancreatic circulation as well as effects of removal of endogenous GABA on pancreatic exocrine secretion should be

determined to verify a physiological role of endogenous GABA in pancreatic exocrine function.

In summary, GABA further elevated pancreatic amylase secretion stimulated by intraduodenal sodium oleate in anesthetized rats. GABA further increased pancreatic amylase secretion stimulated by simultaneous infusion of CCK and secretin. GABA also enhanced CCK-stimulated amylase secretion but was without effect on secretin-stimulated one. Bicuculline markedly reduced the enhancing effects of GABA on pancreatic amylase secretion evoked by sodium oleate as well as CCK plus secretin and CCK alone. Thus it is concluded from the above results that GABA enhances pancreatic amylase secretion evoked by intraduodenal sodium oleate via $GABA_A$-receptors in anesthetized rats. GABA seems to elevate pancreatic amylase secretion evoked by CCK that is released by sodium oleate.

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