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Cholinergic Role on Insulin Action in Exocrine Secretion of the Isolated Rat Pancreas

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=ABSTRACT=

In order to investigate intra-pancreatic cholinergic roles on insulin action in exocrine secretion, the pancreas was isolated from rats and continuously perfused with modified Krebs-Henseleit solution. Intra-arterial infusion of insulin (100 nM) or cholecystokinin (CCK, 14 pM) alone resulted in stimulation of the volume flow and amylase output. Also insulin potentiated the action of CCK in the exocrine secretion. Tetrodotoxin and atropine completely abolished the potentiating action of insulin and CCK as well as the action of insulin alone, but did not change the action of CCK alone. In order to see an effect of intra-pancreatic neural activation on the insulin action, electrical field stimulation (EFS) with parameters of 20 V, 2 msec and 8 Hz was applied to the isolated pancreas for 10 min under 2.5 or 18 mM glucose background. The EFS voltage-dependently elevated the flow rate and amylase output, and potentiated exocrine secretion in 18 mM glucose infusion compared with 2.5 mM glucose. The potentiating effects of EFS and 18 mM glucose were not observed in the streptozotocin-treated pancreas although it was perfused with 18 mM glucose. However, it was restored when the diabetic pancreas was perfused with porcine insulin(100 nM). Tetrodotoxin and atropine inhibited the pancreatic secretion induced by EFS with the background of 18 mM glucose. The results of present investigation indicate that the intra-pancreatic cholinergic tone exerts a stimulatory influence on the action of insulin in pancreatic exocrine secretion of rats.

Key Words: Cholecystokinin, Insulin, Intra-pancreatic neuron, Electrical field stimulation, Pancreatic secretion, Rat

INTRODUCTION

Cholinergic roles in the exocrine pancreas have been suggested from the facts that pancreatic exocrine responses to enteral stimuli or cholecystokinin (CCK) are reduced by vagotomy (Malagelada et al, 1974; Debas et al, 1975; Chey et al, 1979; Fried et al, 1985; Singer et al, 1989) or atropine (Konturek et al, 1972; Singer et al, 1980; Miyasaka & Green 1983; Moriyoshi et al, 1991; Beglinger et al, 1992). Furthermore, the importance of the intra-pancreatic cholinergic neurons in the exocrine pancreas has been suggested by showing that atropine still inhibits exocrine secretion of the extrinsically denervated pancreas in dogs (Singer et al, 1986; Kuvshinoff et al, 1993) and rats (Chariot et al, 1987). Although the secretin-stimulated bicarbonate secretion is reduced by atropine in the extrinsically denervated pancreata of dogs (Singer et al, 1986; Kuvshinoff et al, 1993),

the role of the intra-pancreatic cholinergic neurons in pancreatic exocrine secretion stimulated by gut hormones is unclear at the present time.

It has been reported that potentiation of enzyme secretion in the isolated rat pancreas (Saito et al, 1980; Mueller et al, 1986; Garry et al, 1989) and mouse pancreas (Singh 1985) is occurred by insulin and cholinergic agonists. These results lead to hypothesize that the insulin action on pancreatic exocrine secretion could be affected by the intra-pancreatic cholinergic activity. Insulin has been known to play a very important role in actions of gut hormones on pancreatic exocrine secretion. Insulin potentiates the CCK-stimulated pancreatic exocrine secretion of the isolated rat pancreas (Kanno & Saito 1976; Williams & Goldfine 1985; Park et al, 1993). Insulin is essential for secretin to potentiate the CCK-stimulated pancreatic enzyme secretion of rats (Lee et al, 1996). Depletion of circulating insulin by immunoneutralization results in marked reduction of the pancreatic responses to secretin and CCK-8 of rats (Lee et al, 1990; Lee et al, 1994).

The present study was performed to investigate if the intra-pancreatic cholinergic neurons participating in the potentiating action of insulin on exocrine secretion stimulated by CCK in the isolated perfused rat pancreas.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats, weighing 250~300 g, were used for the present study. The rats were anesthetized with a single intra-peritoneal injection of 25 % urethane (Sigma, USA) at a dose of 0.7 ml/100 g of body weight after 24 hours of fasting but with free access to water. The rats were sacrificed by an overdose of intravenous urethane after isolation of the pancreas.

Potentiating action of insulin on the CCK-stimulated pancreatic exocrine secretion

In order to eliminate extra-pancreatic influences on pancreatic exocrine secretion, the pancreas was isolated according to the method described previously (Penhos et al, 1969; Park et al, 1993). In brief, the pancreas with the duodenum was isolated from the rat and perfused with Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg water) through the celiac and superior mesenteric arteries at a flow rate of 1.2 ml/min. The portal vein was also cannulated to drain the perfusate. The perfusate basically contained 0.1% bovine serum albumin (Sigma), 3% Dextran T-70 (Sigma) and 2.5 mM glucose (Sigma) and oxygenated with 95% O_2 and 5% CO_2. A temperature-controlled organ chamber (37°C) was also constantly supplied with fresh Krebs-Henseleit solution at a flow rate of 0.35 ml/min. After 30 min of the equilibrium period, pancreatic juice secreted within 15 min was sequentially collected during the whole experiment. After that, porcine insulin (Sigma) at a concentration of 100 nM was added in the perfusate for 45 min, and then sulfated CCK-8 (Squibb, USA) at a concentration of 14 pM was also added in the perfusate for 60 min.

Effects of the basal intra-pancreatic neuronal activity on the potentiating action of insulin

Tetrodotoxin (Sigma) at a concentration of 1 μM or atropine (Sigma) at a concentration of 2 μM was added to the perfusate after the basal period until the end of the experiment. While tetrodotoxin was continuously administered to the isolated pancreas, carbamylcholine (Sigma) at a concentration of 50 nM was added to the perfusate from the moment of the CCK addition to the end of the experiment.

Effects of excitation of the intra-pancreatic neurons on the potentiating action of insulin

The intra-pancreatic neurons were excited by application of electrical field stimulation (EFS) to the isolated rat pancreas via a pair of coiled platinum

electrodes immersed in the organ chamber with a distance of 5 cm. Parameters of the EFS were monophasic square wave impulses with 2 msec, 8 Hz and 20 V. EFS was applied from 15 min after the moment of the CCK addition to the end of the experiment in the presence or absence of tetrodotoxin (1 μM) or atropine (2 μM). In order to rule out the possibility that endogenous somatostatin released by EFS attenuates the effect of EFS on the potentiation between insulin and CCK, pertussis toxin (Sigma), a somatostatin inhibitor (Sakamoto et al, 1987; Viguerie et al, 1988), was added to the perfusate at a concentration of 200 ng/ml after the basal period.

Effects of carbamylcholine on the CCK-stimulated amylase secretion in dispersed pancreatic acini

The dispersed pancreatic acini from rats were prepared by the methods reported previously (Williams et al, 1987). Briefly, a pancreas was injected with Krebs-Henseleit bicarbonate solution containing 0.1 mM Ca^{++}, 11.1 mM glucose, 2 mg/ml bovine serum albumin (fraction V; Sigma), 0.1 mg/ml soybean trypsin inhibitor (Sigma), 70~75 U/ml purified collagenase (Sigma) and minimal Eagle's medium amino acid supplement (GIBCO, USA). The injected pancreas was incubated at 37℃ in a shaking incubator with continuous flow of 95% O_2 and 5% CO_2. The pancreatic tissue was then mechanically dissociated and acini were isolated by filtration and centrifugation. Acini were washed with 10 mM N-2-hydroxy-ethylpiperazine-N'-2'-ethanesulfonic acid (HEPES; Sigma) buffered Ringer solution containing 1.28 mM Ca^{++}, 11.1 mM glucose, minimal Eagle's medium amino acid supplement, 0.1 mg/ml soybean trypsin inhibitor and 0.5% bovine serum albumin. Acini were allowed to recover in the HEPES buffered Ringer solution for 60 min in the shaking incubator with continuous flow of 95% O_2 and 5% CO_2. After recovery, acini were centrifuged and resuspended at a density of 20~25 acini/10 μl in the fresh HEPES buffered Ringer solution and then incubated with CCK-8 at a concentration of 10, 30 or 100 pM in the presence or absence of carbamylcholine at a concentration of 50 nM for 30 min at 37°C in the shaking incubator. At the end of the incubation, the acinar suspension was centrifuged at 10,000 x g for 20 sec in an Eppendorf microcentrifuge and amylase activity in the supernatant was determined.

Measurements

The flow rate of pancreatic juice was determined every 15 min by measuring the length of pancreatic juice collected in a microtube, which had a capacity of 3.80 μl/cm. Amylase activity in the pancreatic juice or supernatant was determined by the method of Rick & Stegbauer (1974).

Analysis of data

All values were expressed as mean±SE. Statistical analysis was evaluated by the student's t test. Difference was considered significant if the p value is less than 0.05.

RESULTS

Effects of neuroblockers on the potentiating action of insulin on the CCK-stimulated pancreatic exocrine secretion

The flow rate and amylase output of the isolated rat pancreas during the basal period in which the 2.5 mM glucose was perfused were 1.08±0.34 μl(15 min)$^{-1}$ and 7.17±1.28 U(15 min)$^{-1}$, respectively. As shown in Fig. 1, porcine insulin at a concentration of 100 nM significantly (p<0.05) elevated the pancreatic flow rate and amylase output from the basal levels to 2.76±0.40 μl(15 min)$^{-1}$ and 25.88 ±5.84 U(15 min)$^{-1}$, respectively. When CCK-8 at a concentration of 14 pM was added to the perfusate containing porcine insulin (100 nM), the pancreatic flow rate and amylase output were further increased to 8.69±0.95 μl(15 min)$^{-1}$ and 178.61±15.98 U(15 min)$^{-1}$, respectively. CCK-8 (14 pM) by itself

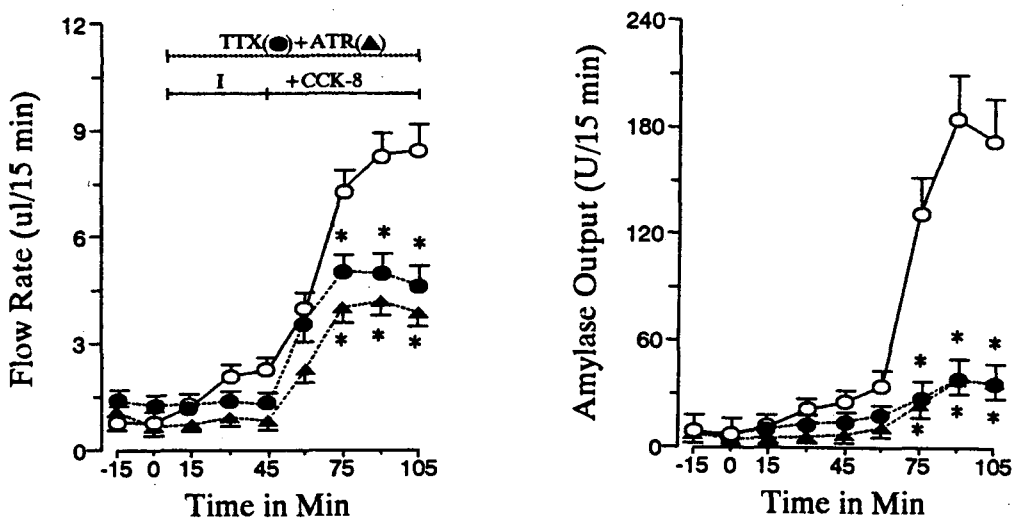

Fig. 1. Effects of neuroblockers on the potentiating actions of insulin on the CCK-stimulated pancreatic exocrine secretion. All pancreata were perfused with porcine insulin (I; 100 nM) and CCK-8 (14 pM) was added at the moment of 45 min. Tetrodotoxin (TTX; 1 μM) or atropine (ATR; 2 μM) was added at the moment of 0 min. Values are presented as means±SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly (P<0.001) different from the corresponding value obtained without the neuroblockers (open circle).

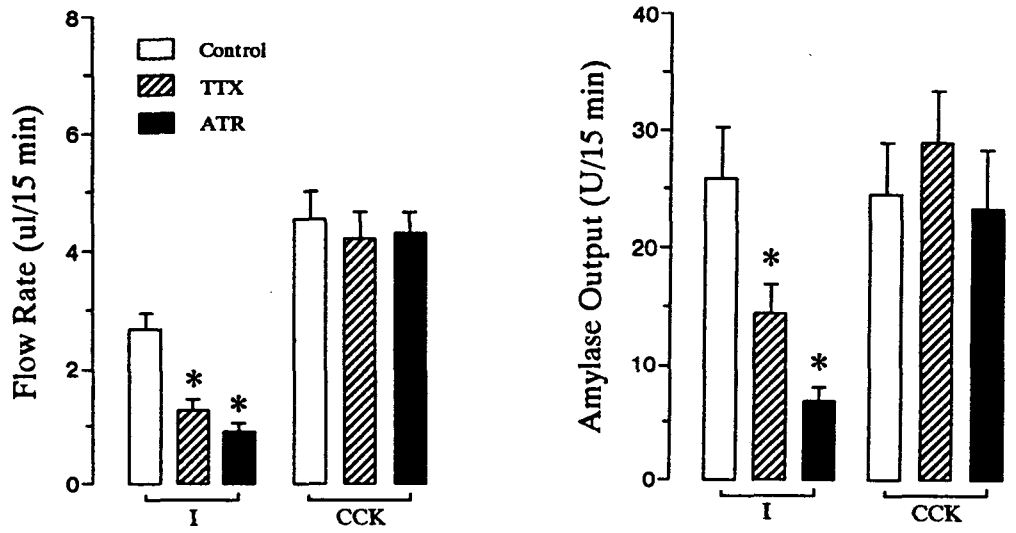

Fig. 2. Effects of neuroblockers on the individual actions of insulin and CCK on the pancreatic exocrine secretion. For details in experimental protocol, see Fig. 1. Values are presented as means±SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly (P<0.05) different from that of the control.

significantly ($p<0.01$) elevated the pancreatic flow rate and amylase output to 4.55 ± 0.53 μl(15 min)$^{-1}$ and 24.53 ± 5.64 U(15 min)$^{-1}$, respectively. Thus, the potentiating action of insulin on the CCK-stimulated pancreatic flow rate and amylase output was also observed in the present study. Tetrodotoxin and atropine, given intra-arterially at concentrations of 1 μM and 2 μM, respectively, completely abolished the potentiating action of insulin on CCK-stimulated pancreatic exocrine secretion. Tetrodotoxin and atropine significantly ($p<0.05$) reduced the pancreatic exocrine responses to insulin alone but did not change the pancreatic responses to CCK alone (Fig. 2). Fig. 3 shows that the cholinergic tone is essential for insulin to potentiate CCK-stimulated pancreatic exocrine secretion in the isolated rat pancreas. Carbamylcholine, an acetylcholine agonist, at a concentration of 50 nM completely resumed the potentiating action of insulin on the CCK-stimulated pancreatic exocrine secretion, which was blocked by tetrodotoxin.

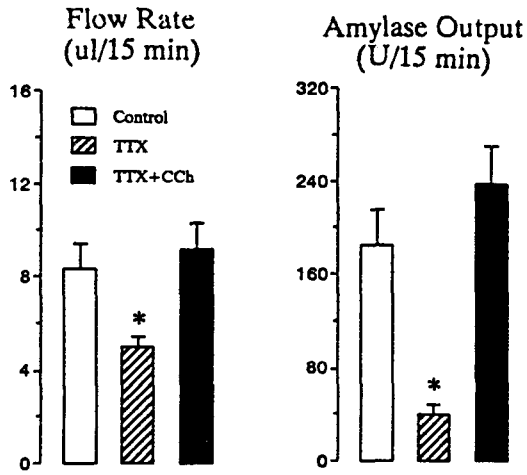

Fig. 3. Effects of carbamylcholine (CCh) on the tetrodotoxin (TTX)-treated pancreatic exocrine secretion. For details in experimental protocol, see Fig. 1. CCh was added to the perfusate at the moment of 45 min. Values are presented as means ±SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly (P<0.01) different from the corresponding value of the control and TTX+CCh.

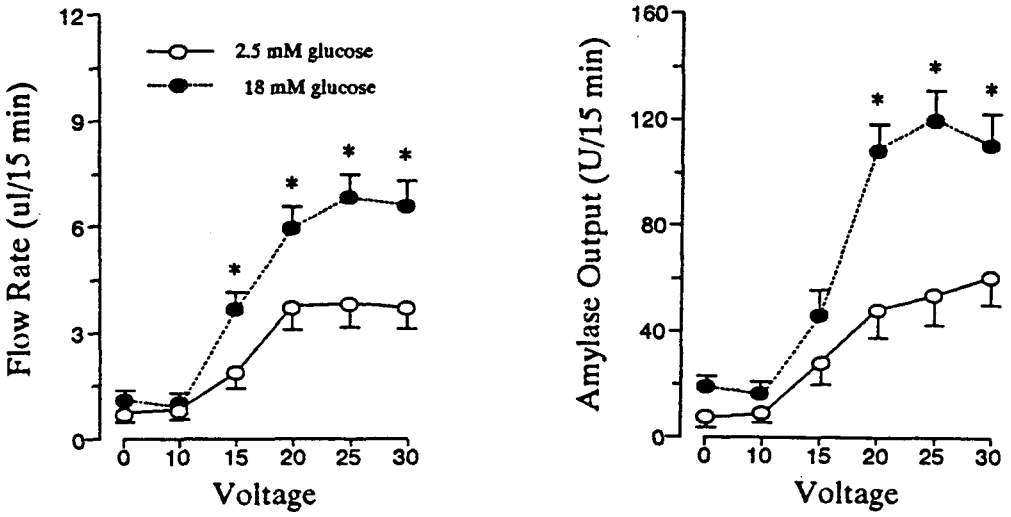

Fig. 4. Voltage-dependent responses of electrical field stimulation (EFS) on the pancreatic exocrine secretion. All pancreata were perfused with the 2.5 mM or 18 mM glucose for 45 min and EFS with various voltages was applied for 10 min. Values are presented as means ±SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly (P<0.01) different from the corresponding value obtained with the 2.5 mM glucose.

Effects of electrical field stimulation on pancreatic exocrine secretion

Fig. 4 illustrates the pancreatic exocrine responses to electrical field stimulation of the isolated rat pancreas. Electrical field stimulation resulted in gradual elevation of the flow rate and amylase output in a voltage-dependent manner. However, the responses to the electrical field stimulation were much greater in the pancreas perfused with the 18 mM glucose than those in the pancreas perfused with the 2.5 mM glucose. The intensity of electrical field stimulation was set at 20 V in the following studies since this intensity appeared to be near maximal to stimulate pancreatic exocrine secretion. As shown in Fig. 5, when electrical field stimulation was applied for 10 min to the isolated pancreas perfused with the 18 mM glucose, the pancreatic flow rate and amylase output were significantly ($p<0.001$) elevated from 1.09 ± 0.11 μl(15 min)$^{-1}$ and 19.30 ± 2.07 U(15 min)$^{-1}$ to 5.97 ± 0.43 μl(15 min)$^{-1}$ and 109.30 ± 15.41 U(15 min)$^{-1}$, respectively. Tetrodotoxin at a concentration of 1 μM and atropine at a concentration of 2 μM significantly ($p<0.001$) reduced the pancreatic responses to the electrical field stimulation. Since the pancreatic exocrine responses to electrical field stimulation were greater in the background of the 18 mM glucose than in the 2.5 mM glucose (Fig. 4), importance of endogenous insulin for electrical field stimulation to elevate the pancreatic exocrine secretion was confirmed in Fig. 6. When electrical field stimulation was applied to the isolated pancreas

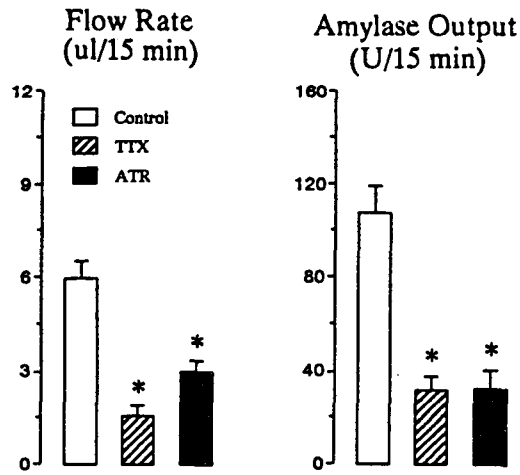

Fig. 5. Effects of neuroblockers on the pancreatic exocrine secretion evoked by electrical field stimulation (EFS). All pancreata were perfused with 18 mM glucose for 45 min and EFS with 20 V was applied for 10 min. Tetrodotoxin (TTX; 1 μM) or atropine (ATR; 2 μM) was added 45 min prior to EFS. Values are presented as means $\pm$SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly ($P<0.01$) different from the corresponding value of the control.

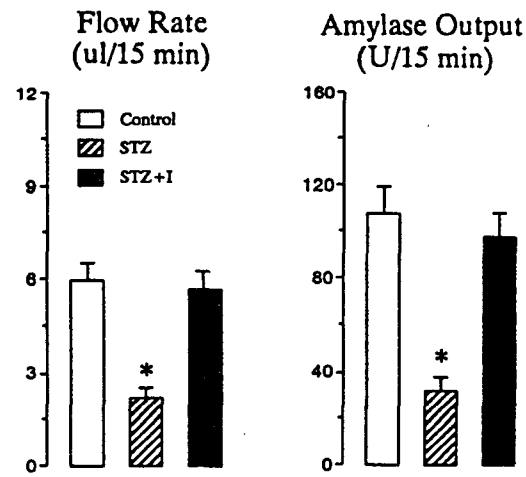

Fig. 6. Effects of electrical field stimulation (EFS) on the exocrine secretion of the streptozotocin (STZ)-treated pancreas. All pancreata were perfused with 18 mM glucose for 45 min and EFS with 20 V was applied for 10 min. Values are presented as means $\pm$SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly ($P<0.01$) different from the corresponding value of the control.

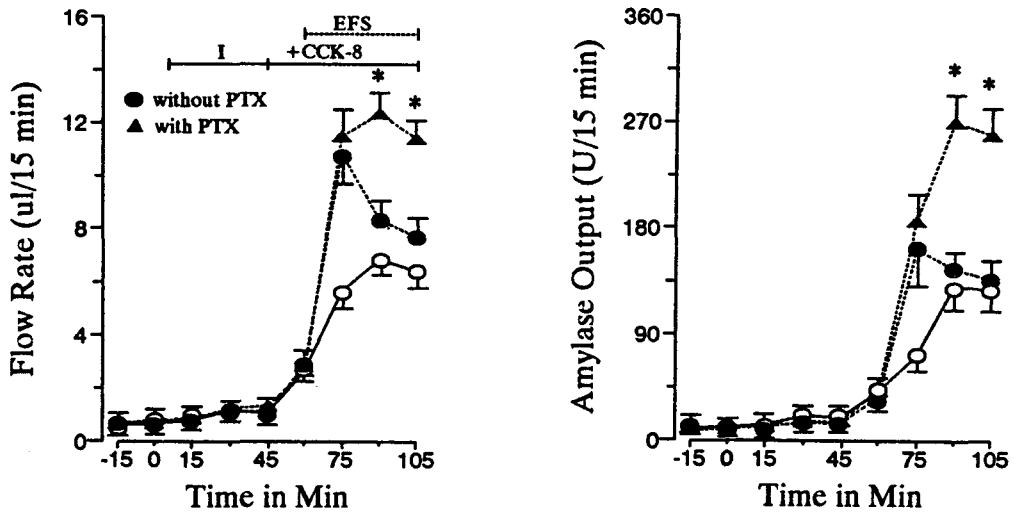

Fig. 7. Effects of electrical field stimulation (EFS) on the potentiating action of insulin on CCK-stimulated pancreatic exocrine secretion. All pancreata were perfused with porcine insulin (100 nM) for 45 min and then CCK-8 (14 pM) was added. EFS with 20 V was applied at 15 min after the addition of CCK-8 for 45 min. Pertussis toxin (PTX; 200 ng/ml) was added at the moment of 45 min prior to CCK-8. Values are presented as means±SE of pancreatic flow rate (left panel) and amylase output (right panel) obtained from seven pancreata. Asterisks indicate the value is significantly (P<0.01) higher than that of the without PIX.

in the background of the 18 mM glucose, the pancreatic flow rate and amylase output in response to the field stimulation were significantly ($p<0.001$) lower in the streptozotocin-treated pancreas than in the normal pancreas. However, the reduced responses to the field stimulation were completely restored when the streptozotocin-treated pancreas was perfused with insulin at a concentration of 100 nM.

Effects of electrical field stimulation on the potentiating action of insulin on the CCK-stimulated pancreatic exocrine secretion

Electrical field stimulation at a intensity of 20 V was applied together with CCK-8 (14 pM) for 45 min in the background of insulin (100 nM). As shown in Fig. 7, the pancreatic flow rate and amylase output in the first 15 min period of the field stimulation were significantly ($p<0.05$) higher than those obtained without the field stimulation. The pancreatic responses in the later 2 periods of the

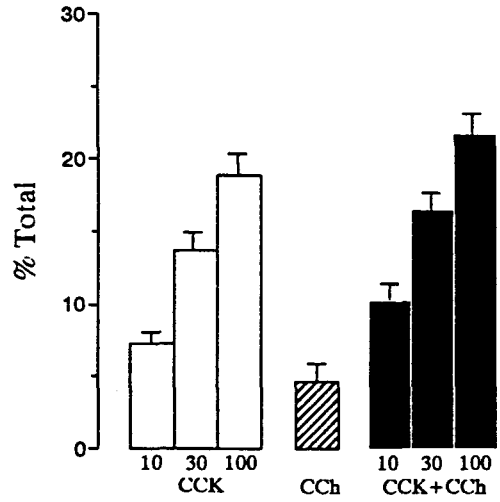

Fig. 8. Effects of carbamylcholine (CCh) on CCK-8-stimulated amylase secretion in the dispersed rat acini. The dispersed acini was incubated with CCK-8 at a concentration of 10, 30, or 100 pM in the presence or absence of CCh at a concentration of 50 nM. Amylase activities in the supernatant are calculated as % total. Values are represented as means±SE of four pancreata.

field stimulation were not different from those obtained without the field stimulation. However, when pertussis toxin was added in the perfusate at a concentration of 200 ng/ml, the pancreatic exocrine responses in the later 2 periods of the field stimulation were significantly ($p<0.01$) higher than not only those obtained without pertussis toxin but also those obtained without the field stimulation.

As shown in Fig. 8, carbamylcholine produced an additional increase in the CCK-8-stimulated amylase secretion in the dispersed rat acini.

DISCUSSION

In the present study, confirming our previous reports (Park et al, 1993; Lee et al, 1996), 100 nM insulin markedly potentiated the pancreatic flow rate and amylase output stimulated by 14 pM CCK-8 in the isolated perfused pancreas of the rat. In order to investigate effects of the basal intra-pancreatic neuronal activity on the potentiation induced by insulin and CCK, the intra-pancreatic neuronal activity was inhibited by tetrodotoxin, a well-known neurotoxin, or excited by electrical field stimulation. Tetrodotoxin blocked the potentiating action of insulin on CCK-8-stimulated pancreatic exocrine secretion. Electrical field stimulation of the intra-pancreatic neurons resulted in a further elevation of the pancreatic exocrine secretion potentiated by insulin and CCK-8. Since the pancreas was totally isolated from the rat and the perfusate was not recirculated in this study, extra-pancreatic, including neural and other hormonal, influences on pancreatic exocrine secretion were completely eliminated. Thus, the results of this study indicate that the potentiating action of insulin to the CCK-stimulated pancreatic exocrine secretion is dependent on the basal intra-pancreatic neuronal activity.

Among the intra-pancreatic neurons, the cholinergic neurons were thought to exert an excitatory influence on pancreatic exocrine secretion. Urecholine, a cholinergic agonist, as well as electrical field stimulation released amylase from the rat pancreatic segments and their effects were inhibited by atropine (Varga et al, 1990). Atropine still inhibited the basal or secretin-stimulated exocrine secretion of the extrinsically denervated pancreas in rats (Chariot et al, 1987) and dogs (Singer et al, 1986; Kuvshinoff et al, 1993). Thus, the role of the intra-pancreatic cholinergic activity in the potentiating action of insulin to the CCK-stimulated pancreatic exocrine secretion was investigated in this study. Atropine, a muscarinic receptor antagonist, blocked the potentiating action of insulin and CCK-8. Furthermore, carbamylcholine, an acetylcholine agonist, completely restored the tetrodotoxin-blocked potentiating action of insulin and CCK-8. The results clearly show that the potentiating action of insulin to the CCK-stimulated pancreatic exocrine secretion is dependent on the basal excitatory tone of the intra-pancreatic cholinergic neurons. The present study also demonstrates that atropine as well as tetrodotoxin reduces pancreatic exocrine secretion evoked by insulin alone but neither tetrodotoxin nor atropine changes pancreatic exocrine secretion stimulated by CCK-8 alone. These results indicate that the insulin action in the pancreatic exocrine secretion is dependent on the basal intra-pancreatic cholinergic tone while the CCK action is not. Thus, we assume that the basal intra-pancreatic cholinergic tone exerts the facilitatory influence on the potentiation between insulin and CCK in pancreatic exocrine secretion by promoting the insulin action rather than the CCK action.

Although it has been reported that potentiation occurred by insulin and acetylcholine agonists in enzyme secretion of the isolated rat pancreas (Saito et al, 1980; Mueller et al, 1986; Garry et al, 1989) and of the mouse pancreatic segments (Singh 1985), the intra-pancreatic cholinergic dependency of the insulin action in pancreatic exocrine secretion is unclear at the present time. In the present study, atropine and tetrodotoxin reduced the pancreatic flow rate and amylase output stimulated by insulin. The excitation of the intra-pancreatic neurons by electrical field stimulation resulted in augmentation

of the insulin-stimulated pancreatic exocrine secretion. The effects of electrical field stimulation on the insulin-stimulated pancreatic exocrine secretion were reduced by atropine. The results indicate that the insulin action in the pancreatic exocrine secretion is very much dependent on the intra-pancreatic cholinergic activity.

In the present investigation, atropine did not change pancreatic exocrine secretion induced by CCK-8 alone but reduced pancreatic exocrine secretion stimulated by a mixture of CCK-8 and insulin. These results suggest that atropine does not affect pancreatic exocrine secretion stimulated by CCK alone but inhibits the potentiated pancreatic exocrine secretion induced by insulin and CCK. Slightly different results have been reported by using the same model as ours, in which atropine and pirenzepine inhibited the CCK-stimulated pancreatic juice flow, but not enzyme secretion, although 100 pM of CCK was perfused in the background of 8.3 mM glucose (Otsuki et al, 1987). Thus, we further confirmed the interaction of CCK and the intra-pancreatic cholinergic activity. Excitation of the intra-pancreatic cholinergic neurons by electrical field stimulation resulted in only an additional increase in the CCK-stimulated pancreatic flow rate and amylase output in the isolated rat pancreas. Carbamylcholine also produced an additional increase in the CCK-8-stimulated amylase secretion in the dispersed rat acini. The additional interaction in pancreatic exocrine secretion between CCK and carbamylcholine or electrical field stimulation might have occurred because CCK and acetylcholine agonist have the same intra-cellular pathway (Gardner & Jenssen 1980). Thus, the CCK action in the pancreatic exocrine secretion appears not to be modulated by the intra-pancreatic cholinergic activity. However, the cholinergic dependency of the CCK action has already been suggested by showing that atropine inhibits pancreatic responses to physiological doses of CCK-8 in conscious rats (Moriyoshi et al, 1991) and anesthetized rats (Li & Owyang 1993). The discrepancy is likely to be related to other neural or hormonal factors, particularly insulin, may affect the CCK action in pancreatic exocrine secretion *in vivo* situations. It has been reported that pancreatic exocrine secretion stimulated by a liquid meal as well as secretin and CCK is blocked by anti-insulin antibody in conscious rats (Lee et al, 1990).

In summary, the potentiation by insulin and CCK in pancreatic exocrine secretion was affected by the intra-pancreatic cholinergic activity. The insulin actions but not the CCK actions were modified by the intra-pancreatic cholinergic activities. It has been concluded from the above results that the intra-pancreatic cholinergic neurons exert the excitatory influences on the potentiation between insulin and CCK by promoting the insulin action in pancreatic exocrine secretion of rats.

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REFERENCES

Beglinger C, Hildebrand P, Adler G, Werth B, Luo H, Delco F & Gyr K (1992) Postprandial control of gallbladder contraction and exocrine pancreatic secretion in man. *Eur J Clin Invest* **22**, 827-834

Chariot J, De la tour J, Anglade P & Roze C (1987) Cholinergic mechanisms in the pancreas after extrinsic denervation in the rat. *Am J Physiol* **252**, G755-G761

Chey WY, Kim MS & Lee KY (1979) Influence of the vagus nerve on the release and action of secretin in the dog. *J Physiol* **293**, 435-446

Debas HT, Konturek SJ & Grossman MI (1975) Effect of extragastric and truncal vagotomy on pancreatic secretion in the dog. *Am J Physiol* **228**, 1172-1177

Fried GM, Ogden WD, Sakamoto T, Greeley GH & Thompson JC (1985) Experimental evidence for a vagally mediated and cholecystokinin independent enteropancreatic reflex. *Ann Surg* **202**, 69-74

Gardner JD & Jenssen RT (1980) Receptor for secretagogues on pancreatic acinar cells. *Am J Physiol*

238, G63-G66

Garry DJ, Garry MG, Williams JA, Mahoney WC & Sorenson RT (1989) Effect of islet hormones on amylase secretion and localization of somatostatin binding sites. *Am J Physiol* **256**, G897-G904

Kanno T & Saito A (1976) The potentiating influences of insulin on pancreozymin-induced hyperpolarization and amylase release in the pancreatic acinar cell. *J Physiol* **261**, 505-521

Konturek SJ, Tassler J & Obtulowics W (1972) Effect of atropine on pancreatic responses to endogenous and exogenous cholecystokinin. *Am J Dig Dis* **17**, 911-917

Kuvshinoff BW, Demar AR, James L, Mcfadden DW & Fink AS (1993) Effect of pancreatic denervation and atropine on the pancreatic response to secretin. *Pancreas* **5**, 609-614

Lee YL, Kwon HY, Park HS, Lee TH & Park HJ (1996) The role of insulin in the interaction of secretin and cholecystokinin in exocrine secretion of the isolated perfused rat pancreas. *Pancreas* **12**, 58-63

Lee KY, Lee YL, Kim CD, Chang T-M & Chey WY (1994) Mechanism of action of insulin on pancreatic exocrine secretion in perfused rat pancreas. *Am J Physiol* **267**, G207-G212

Lee KY, Zhou L, Ren XS, Chang T-M & Chey WY (1990) An important role of endogenous insulin on exocrine pancreatic secretion in rats. *Am J Physiol* **258**, G268-G274

Li Y & Owyang C (1993) Vagal afferent pathway mediate physiological action of cholecystokinin on pancreatic enzyme secretion. *J Clin Invest* **92**, 418-424

Malagelada JR, Go VLW & Sumerskill HJ (1974) Altered pancreatic and biliary function after vagotomy and pyloroplasty. *Gastroenterology* **66**, 22-27

Miyasaka K & Green GM (1983) Effect of atropine on rat basal pancreatic secretion during return or diversion of bile-pancreatic juice. *Proc Soc Exp Biol Med* **174**, 187-192

Moriyoshi Y, Shiratori K, Watanabe S & Takeuchi T (1991) Potentiating effect of CCK and secretin on rat exocrine pancreas and its cholinergic dependence. *Pancreas* **5**, 603-608

Mueller MK, Scheck T, Demol P & Goebell H (1986) Interaction of acetylcholine and gastric inhibitory polypeptide on endocrine and exocrine rat pancreatic secretion: Augmentation of acetylcholine-induced amylase and volume secretion by the insulinotropic action of gastric inhibitory polypeptide. *Digestion* **33**, 45-52

Otsuki M, Okabayashi Y, Nakamura T, Fujii M, Oka T, Tani S & Baba S (1987) Inhibitory effects of pirenzepine on cholecystokinin and secretin stimulation on exocrine and endocrine rat pancreas. *Dig Dis Sci* **32**, 1136-1144

Park HJ, Lee YL & Kwon HY (1993) Effects of pancreatic polypeptide on insulin action in exocrine secretion of isolated rat pancreas. *J Physiol (London)* **463**, 421-429

Penhos JC, Wu CH, Basebe JC, Lopez N & Wolf FW (1969) A rat pancreas-small gut preparation for the study of intestinal factor(s) and insulin release. *Diabetes* **18**, 733-738

Rick W & Stegbauer HP (1974) α-Amylase; Measurement of reducing groups. *In Methods of Enzymatic Analysis*, Bergereyer HY, (2nd ed), vol 2, Verlag Chemie, Weinheim p885-915

Saito A, Williams JA & Kanno T (1980) Potentiation by insulin of the acetylcholine-induced secretory response of the perfused rat pancreas. *Biomed Res* **1**, 101-103

Sakamoto C, Matozaki T, Nagao M & Baba S (1987) Coupling of guanine nucleotide inhibitory protein to somatostatin receptors on pancreatic acinar membranes. *Am J Physiol* **253**, G308-G314

Singer MV, Niebel W, Jansen JG, Hoffmeister D, Gotthold S, Goebell H & Lamers CB (1989) Pancreatic secretory response to intravenous caerulein and intraduodenal tryptophan studies: before and after stepwise removal of the extrinsic nerves of the pancreas in dogs. *Gastroenterology* **96**, 925-934

Singer MV, Niebel W, Kniesburgs S, Hoffmeister D & Goebell H (1986) Action of atropine on the pancreatic secretory response to secretin before and after cutting the extrinsic nerves of the pancreas in dogs. *Gastroenterology* **90**, 353-361

Singer MV, Solomon TE & Grossman MI (1980) Effect of atropine on secretion from intact and transplanted pancreas in dogs. *Am J Physiol* **238**, G18-G22

Singh J (1985) Mechanism of action on acetylcholine-evoked amylase secretion in the mouse pancreas. *J Physiol (London)* **255**, 469-482

Varga G, Papp M & Vizi ES (1990) Cholinergic and adrenergic control of enzyme secretion in isolated

rat pancreas. *Dig Dis Sci* **35**, 501-507

Viguerie N, Tahiri-Jouti N, Esteve JP, Clerc P, Logsdon C, Svoboda M, Susini C, Vaysse N & Ribet A (1988) Functional somatostatin receptors on a rat pancreatic acinar cell line. *Am J Physiol* **255**, G113-G120

Williams JA & Goldfine ID (1985) The insulin-pancreatic acinar axis. *Diabetes* **34**, 980-986

Williams JA, Korc M & Dormer RL (1987) Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini *Am J Physiol* **235**, E517-E524