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Mechanism of Action of Pancreatic Polypeptide (PP) on Pancreatic Exocrine Secretion in Isolated Rat Pancreas

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Aim of this study was to investigate if pancreatic polypeptide (PP) reduced the insulin action via the intra-pancreatic cholinergic nerves in the isolated rat pancreas. The pancreas was isolated from rats and perfused with intra-arterial infusion of modified Krebs-Henseleit solution containing 2.5 mM glucose at a flow rate of 1.2 ml/min. Simultaneous intra-arterial infusion of insulin (100 nM) resulted in potentiation of the pancreatic flow rate and amylase output which were stimulated by cholecystokinin (CCK, 14 pM). These potentiating actions of insulin on the CCK-stimulated pancreatic exocrine secretion were completely abolished by administration of rat PP. Vesamicol, a potent inhibitor of vesicular acetylcholine storage, and tetrodotoxin (TTX) also significantly reduced the combined actions of insulin and CCK. Administration of carbamylcholine, an acetylcholine agonist, completely restored the vesamicol- or TTX-induced inhibition of the potentiation between insulin and CCK. Also rat PP failed to attenuate the restoring effect of carbamylcholine. Electrical field stimulation (15-30 V, 2 msec and 8 Hz) resulted in a significant increase in the pancreatic flow rate and amylase output in voltage-dependent manner. Effects of electrical field stimulation were augmented by endogenous insulin. Rat PP also suppressed the pancreatic exocrine secretion stimulated by electrical field stimulation. These observations strongly suggest that PP inhibits the potentiating actions of insulin on CCK-stimulated pancreatic exocrine secretion by suppression of the intra-pancreatic cholinergic activity in the isolated rat pancreas.

Key Words: Pancreatic polypeptide, Electrical field stimulation, Cholecystokinin, Insulin, Pancreatic secretion, Rat.

INTRODUCTION

It has been well known that pancreatic polypeptide (PP) inhibits pancreatic exocrine secretion in humans (Greenberg et al, 1979; Konturek et al, 1982), dogs (Lin et al, 1977; Taylor et al, 1979; Shiratori et al, 1985), and rats (Kim & Case, 1980; Louie et al, 1985; Park et al, 1993). However, the inhibitory mechanism remains to be clarified further at the present time. It has been reported that PP inhibits amylase release by directly acting with the pancreatic acini (Raimond & DeJoseph, 1986). In contrast, indirect actions of PP have suggested that PP failed to produce change in amylase secretion induced by CCK alone in the dispersed rat pancreatic lobule (Louie et al, 1985) and in the isolated cat pancreas (Kim & Case, 1980). Furthermore, binding sites of PP to the acinar cells were not observed (Louie et al, 1985). We also have previously reported, in the isolated rat pancreas, that rat PP inhibits pancreatic exocrine secretion by reducing the potentiating actions of insulin on cholecystokinin (CCK)-stimulated pancreatic exocrine secretion (Park et al, 1993). It has also been reported that intra-pancreatic cholinergic activity plays a stimulatory role in pancreatic exocrine secretion (Lingard & Young, 1983) and that PP plays the role as an antagonist of muscarinic cholinergic receptors (Pan et al, 1987). It has been reported that insulin potentiates CCK-stimulated pancreatic exocrine secretion (Saito et al, 1980; Park et al, 1993) and insulin also potentiates acetylcholine-stimulated (Saito et al, 1980) or carbamylcholine -stimulated pancreatic exocrine secretion in the

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isolated rat pancreas (Garry et al, 1989). Therefore, there is a possible interaction of PP, insulin and intra-pancreatic cholinergic nerves. However, the mechanism by which PP modulates the intra-pancreatic activity in the pancreas is still unclear.

Thus the purpose of this study was to investigate the possible role of PP in reducing the insulin action via the intra-pancreatic cholinergic nerves in the isolated rat pancreas.

METHODS

Experimental animals

In total, ninety-four male Sprague-Dawley rats, weighing 250~300 g, were anesthetized with a single intraperitoneal injection of 25% urethane (Sigma, USA) at a dose of 0.7 ml/100 g of body weight. The rats were sacrificed by an intravenous overdose of urethane after isolation of the pancreas. Each rat was put on a 24 hours fast before the experiment, but was allowed to drink water freely.

Preparation of isolated perfused pancreas

The isolated pancreata were prepared according to the method described previously (Kanno & Saito 1976; Park et al, 1993). In brief, the pancreas with the duodenum was isolated and perfused with Krebs-Henseleit solution (pH 7.4, 305 m Osmol/kg water) through the celiac and superior mesenteric arteries at a flow rate of 1.2 ml/min by multistaltic pump (Haakebuchler, USA). The portal vein was also cannulated to drain the perfusate. The perfusate contained 0.1% bovine serum albumin (Sigma, USA), 3% dextran T-70 (Sigma, USA) and 2.5 mM glucose (Sigma, USA) and was oxygenated with 95% O_2 and 5% CO_2. For collection of the pancreatic juice, PE-10 tubing (Clay Adams, USA) was placed into the pancreatic duct and the hepatic end of the common bile duct was ligated. The isolated pancreas, including duodenum, was placed in a temperature-controled chamber at 37°C. The organ chamber was also constantly supplied with fresh Krebs-Henseleit solution at a rate of 0.35 ml/min. After 30 min of the equilibriation period in the organ chamber, pancreatic juice secreted within 15 min was sequentially collected during the whole period of the experiment.

Effects of rat PP on actions of insulin and CCK

In the control experiment, the isolated pancreas was perfused with Krebs-Henseleit solution containing 2.5 mM glucose for 45 min, and then porcine insulin (Sigma, USA) and sulfated CCK-8 (Squibb, USA) were added to the perfusate during another 60 min at concentrations of 100 nM and 14 pM, respectively. For the purpose of observing effects of PP on the interaction of insulin and CCK-8, the isolated pancreas was perfused with rat PP (Peninsula, USA; 10 pM) or somatostatin (Sigma, USA; 1 nM) under 2.5 mM glucose background, and then insulin and CCK-8 were added to the perfusate. In order to see a cholinergic influence on the actions of insulin and CCK-8, vesamicol (RBI, USA; 10 μM), a potent inhibitor of vesicular acetylcholine storage, and tetrodotoxin (Sigma, USA; 1 μM) were administered to the perfusate.

Effects of PP on actions of electrical field stimulation

To observe the effect of excitation of the intra-pancreatic neurons in pancreatic exocrine secretion, electrical field stimulation (EFS) was applied to the isolated rat pancreas via a pair of coiled platinum electrodes immersed in a temperature-controled organ chamber with a distance of 5 cm. Parameters of the EFS were monophasic square wave impulses with 2 msec, 8 Hz and 10-30 V. EFS was applied for 10 min after the equilibriuation period in the presence or absence of PP.

In order to see if endogenous insulin potentiates the action of EFS in pancreatic flow rate and amylase output, 18 mM glucose was perfused for 45 min, instead of 2.5 mM glucose, and then EFS was applied to the isolated pancreas. Various intensities (10, 15, 20, 25 and 30 V) were given to the pancreas. For the purpose of observing the effect of PP on the action of EFS (15 V), various doses of PP were 10, 30 and 100 nM administrated in Krebs-Henseleit solution containing 18 mM glucose PP was administered for 45 min after basal period and then EFS applied for 45 min.

Measurements

The flow rate of the pancreatic juice was determined every 15 min by measuring the length of microbore tubing, which had a capacity of 3.8 μl/cm. Amylase activity in pancreatic juice was determined by the method of Rick & Stegbauer (1974).

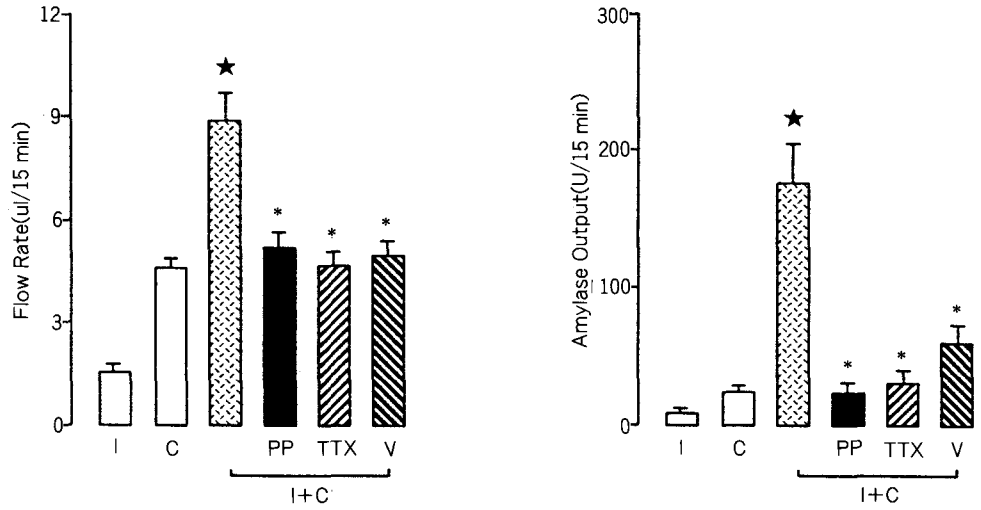

Fig. 1. Effects of pancreatic polypeptide (PP), tetrodotoxin (TTX) and vesamicol (V) on the action of insulin (I) and CCK (C) in pancreatic flow rate and amylase output of the isolated rat pancreas. Each value represents mean ± SE of seven pancreata. Stars indicate that $P<0.05$ compared with pancreatic responses to the summation value of insulin and CCK alone. Asterisks indicate that $P<0.05$ compared with pancreatic responses to the values of combination of insulin and CCK.

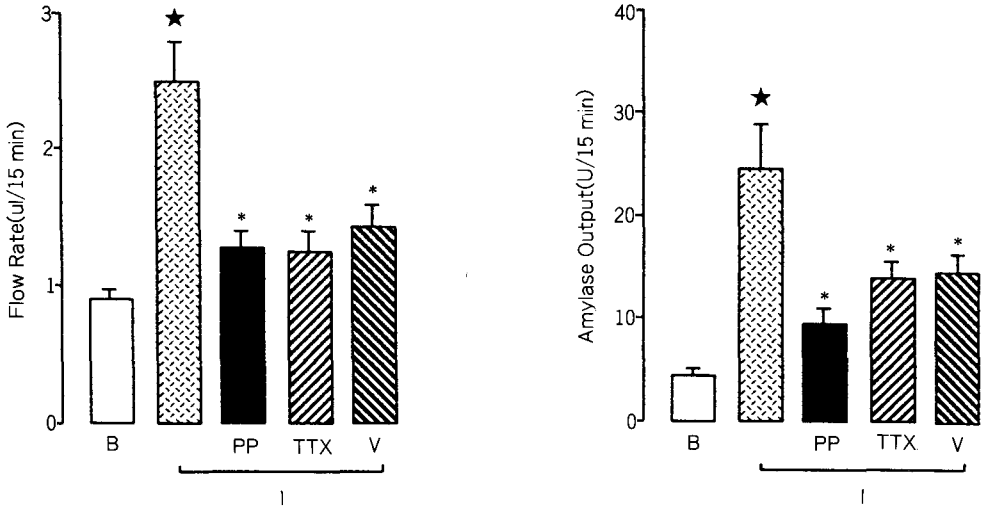

Fig. 2. Effects of pancreatic polypeptide (PP), tetrodotoxin (TTX) and vesamicol (V) on the action of insulin alone in pancreatic flow rate and amylase output of the isolated rat pancreas. Each value represents mean ± SE of seven pancreata. Stars indicate that $P<0.05$ compared with pancreatic response to basal (B). Asterisks indicate that $P<0.05$ compared with pancreatic response to insulin (I) alone.

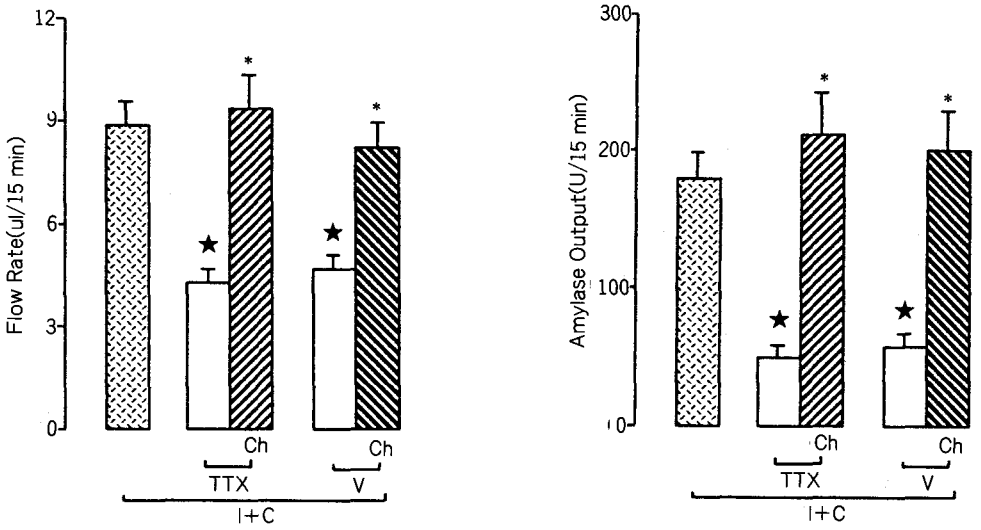

Fig. 3. Effects of carbamylcholine (Ch) on the action of insulin (I) and CCK (C) inhibited by tetrodotoxin (TTX) and vesamicol (V). Each value represents mean±SE of seven pancreata. Stars indicate that P<0.05 compared with pancreatic response to the action of insulin and CCK. Asterisks indicate that P<0.05 compared with pancreatic response to the action of tetrodotoxin and vesamicol.

Analysis of data

All values are expressed as mean ±SE. Student's *t* test or paired *t* test was used for statistical analysis of the data. Values of *p* less than 0.05 were considered significant.

RESULTS

Effects of rat PP on pancreatic secretion stimulated by insulin and/or CCK-8

As shown in Fig. 1, when the isolated rat pancreas was perfused with exogenous insulin or CCK-8, pancreatic flow rate and amylase output were 1.48± 0.17 or 4.55±0.53 μl/15 min and 16.19±5.78 or 24.53±5.63 U/15 min, respectively. However, when the isolated rat pancreas was perfused with exogenous insulin and CCK, the pancreatic flow rate and amylase output was further elevated to 8.99±0.95 μl/15 min and 181.87±29.27 U/15 min, respectively. Since the values are much higher than sums of insulin and CCK-8 alone in pancreatic flow rate and amylase output, potentiation occurred in both parameters. The potentiation effects of insulin on CCK action in the isolated pancreas was significantly (P<0.01) inhibited by administration of rat PP, tetrodotoxin(TTX) and

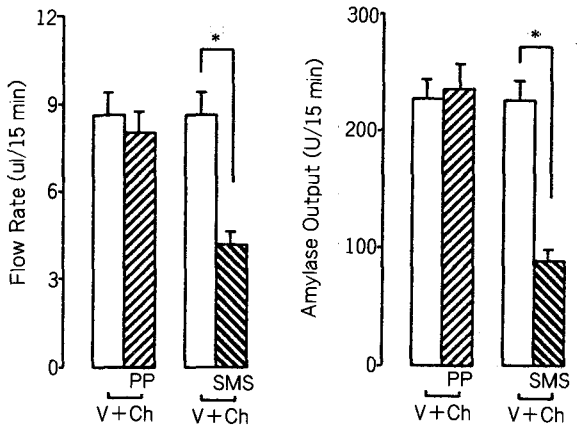

Fig. 4. Effects of pancreatic polypeptide (PP) and somatostatin (SMS) on the combined action of vesamicol (V) and carbamylcholine (Ch) in pancreatic flow rate and amylase output stimulated by insulin and CCK. Each value represents mean±SE of six pancreata. Asterisks indicate significant (P<0.05) differences between without SMS and with SMS.

vesamicol. The pancreatic flow rate decreased from 8.99±0.95 to 5.04±0.60, 4.72±0.12 and 4.91±0.35 μl/15 min, respectively, and amylase output was also suppressed from 181.87±29.27 to 32.80±3.93, 41.25 ±10.21 and 71.9±19.6 U/15 min, respectively. Rat

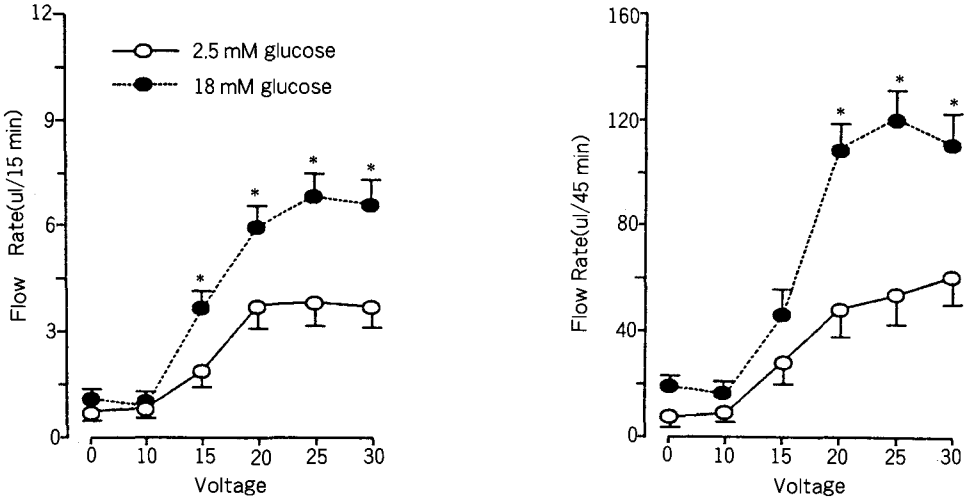

Fig. 5. Effects of endogenous insulin on the action of electrical field stimulation (EFS) in pancreatic flow rate and amylase output. All pancreata were perfused with the 2.5 mM or 18 mM glucose for 45 min and then EFS with various voltages were applied for 10 min. Six pancreata were used for the experiment. Vertical bars represent mean ±SE. Asterisks indicate the value is significantly ($P<0.05$) different from the corresponding values obtained with 2.5 mM glucose.

PP as well as TTX and vesamicol also abolished the action of insulin alone in the pancreatic flow rate and amylase output (Fig. 2). As shown in Fig. 3, when carbamylcholine was added in the perfusate with insulin, CCK and vesamicol or TTX, the combined action of insulin and CCK inhibited by vesamicol and TTX was reversed. The values are not significant compared to the actions of insulin and CCK. Rat PP failed to attenuate the pancreatic exocrine secretion that was reversed from the vesamicol- and TTX-induced inhibition by carbamylcholine. However, as shown in Fig. 4, somatostatin significantly suppressed ($P<0.01$) the pancreatic flow rate and amylase output reversed by carbamylcholine (flow rate: 4.41±0.30 μl/15 min, amylase output: 91.4±14.4 U/15 min).

Effects of rat PP on electrical field stimulation in pancreatic secretion

As shown in Fig. 5, endogenous insulin significantly ($P<0.05$) potentiated the actions of electrical field stimulation in pancreatic flow rate and amylase output. When the intensity of electrical field stimulation was increased to 10, 15, 20, 25 and 30 V under 18 mM glucose background as well as 2.5 mM glucose background, pancreatic flow rate and amylase output elevated in voltage-dependent manner (Fig. 6).

When electrical field stimulation was applied with

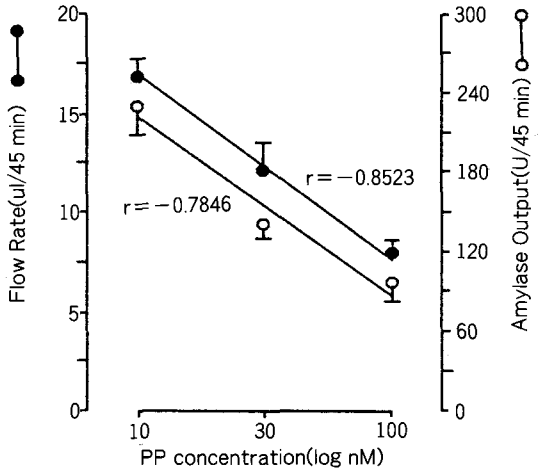

Fig. 6. Effects of three different doses of pancreatic polypeptide (PP) on the action of electrical field stimulation (EFS) with endogenous insulin background in pancreatic flow rate and amylase output. All pancreata were perfused with 18 mM glucose for 45 min and then EFS with 15 V was applied for 45 min. Each value represents mean±SE of seven pancreata.

PP at concentrations of 10, 30 and 100 nM, pancreatic flow rate and amylase output decreased in dose-dependent manner (Fig. 6). The correlation coefficients of the flow rate and amylase output were

0.8523 and 0.7846, respectively. The correlations were statistically significant ($P<0.05$).

DISCUSSION

In the present study, we confirmed our previous results that insulin potentiates CCK-stimulated pancreatic secretion (Park et al, 1993). It is also in close agreement with other reports (Garry et al, 1989; Kanno & Saito, 1976; Matsushita et al, 1994). Rat PP completely abolished the potentiating actions of insulin on the CCK-stimulated pancreatic exocrine secretion in the isolated rat pancreas. Furthermore, rat PP inhibits pancreatic exocrine secretion induced by insulin alone but it does not affect pancreatic secretion stimulated by CCK alone. Similar results were obtained that vesamicol, a potent inhibitor of vesicular acetylcholine storage, and TTX reduced the combined actions of insulin and CCK-8. These results indicate that PP exert an inhibitory role in pancreatic secretion by modulating the insulin action. Furthermore, carbamylcholine, an acetylcholine agonist, completely restored the TTX- and vesamicol-induced inhibition of the potentiation between insulin and CCK-8. These results clearly show that intra-pancreatic cholinergic activity plays an important role in pancreatic exocrine secretion. Rat PP failed to attenuate the pancreatic exocrine secretion which restored the vesamicol-induced inhibition by carbamylcholine. However, somatostatin significantly suppressed the pancreatic secretion in pancreatic exocrine secretion that was reversed from the vesamicol-induced inhibition by carbamylcholine. Taken together, these results suggest that 1) rat PP inhibits the potentiation action of insulin on CCK-stimulated pancreatic exocrine secretion by suppressing the insulin action via intra-pancreatic cholinergic activity in the isolated rat pancreas and 2) an action mechanism of PP differs from somatostatin, one of islet hormones.

In the present study the administration of carbamylcholine at a concentration of 50 nM that is close to the lower for a response restore the TTX- and vesamicol-induced inhibition of the potentiation between insulin and CCK-8. Since Garry et al (1989) reported that insulin potentiated the pancreatic exocrine secretion stimulated by carbamylcholine, mimicked concentration should be required for prevention of this phenomena to find the effect of PP in this experimental design. Furthermore, PP failed to attenuate the pancreatic exocrine secretion which restored the vesamicol-induced inhibition even if carbamylcholine was administered. These results also mean that the concentration of carbamylcholine is a mimicked dose. It has been reported that PP inhibited acetylcholine release stimulated by potassium ions in the isolated pancreatic lobule (Jung et al, 1987), and that a high dose of PP (more than 50 μg/kg) reduced insulin release in conscious rats (Gettys et al, 1991). However, a very low dose of PP (10 pM) was used in this study. The dose of PP also inhibited pancreatic exocrine secretion stimulated by exogenous insulin alone and completely blocked the potentiation action of exogenous insulin on CCK-stimulated pancreatic secretion in the present study. Thus, we suspect that PP inhibits the pancreatic secretion by suppression of the actions of insulin via modulation of activity of the intra-pancreatic cholinergic nerve rather than acetylcholine release or insulin release. Even if PP inhibited pancreatic enzyme secretion by presynaptic modulation of acetylcholine release (Varga et al, 1990), it is still unclear at the present time that PP modulates the cholinergic activity in the pancreas. Therefore, in order to prevent other influences such as adrenergic activity, we used TTX. In the present experiment, we used very high doses of rat PP at concentrations of 10 - 100 nM to inhibit the action of electrical field stimulation. However, since the pancreas has the insulo-acinar portal system in rats (Lifson et al, 1985; Williams & Goldfine, 1985) and a single pass perfusion system was used in this study, PP released from the islet must act on the acinar cell via the insulo-acinar portal system. Therefore, it is a possibility that concentrations of PP acting in the acinar cell is much higher than that of PP in systemic circulation. When the pancreatic lobule was applied with electrical field stimulation, pancreatic amylase output increased (Lingard & Young, 1983) and this effect of electrical field stimulation was blocked by atropine (Varga et al, 1990). In this experiment, electrical field stimulation also significantly elevated pancreatic flow rate and amylase output in the isolated rat pancreas. Our results were in close agreement with Lingard and Young's report (Lingard & Young, 1983). These results indicate that intra- pancreatic cholinergic nerves play a stimulatory role in pancreatic exocrine secretion by direct action. The most important finding of the present investigation is that insulin potentiates the actions of electrical field stimulation and PP inhibits the potentiating actions of insulin on electrical field stimulation in a dose-de-

pendent manner. Taken together, these results suggest that 1) intra-pancreatic cholinergic neurons directly act as a stimulatory factor in pancreatic secretion and 2) an islet hormone such as PP may act on the intra-pancreatic cholinergic neurons. Indirect actions of PP have been suggested from several groups. PP failed to produced change in amylase secretion induced by CCK alone in the dispersed rat pancreatic lobule (Louie et al, 1985) and the incubated rat pancreas (Lin et al, 1977) as well as in the isolated cat pancreas (Kim & Case, 1980). We also reported that PP did not alter pancreatic secretion stimulated by CCK alone in the isolated rat pancreas (Park et al, 1993). More sound evidence was presented by Louie et al (1985) that a binding site of PP to the acinar cells was not observed. 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 inhibitory action of PP in pancreatic exocrine secretion were shown by suppression of the intra-pancreatic neuronal activity. In the most current report, however, PP acts in the dorsal motor nucleus to modulate vagal tone on the pancreas, thereby inhibiting pancreatic secretion (Okumura et al, 1994). Thus, to find all of the action mechanisms of PP, more studies should be performed at the present time.

In conclusion, PP inhibits the potentiating action of insulin on CCK-stimulated pancreatic exocrine secretion by suppressing the intra-pancreatic cholinergic activity in the isolated perfused rat pancreas.

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