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EFFECTS OF PANCREATIC POLYPEPTIDE ON INSULIN ACTION IN EXOCRINE SECRETION OF ISOLATED RAT PANCREAS

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SUMMARY

1. Effects of pancreatic polypeptide (PP) on insulin action in pancreatic exocrine secretion was investigated by using an isolated rat pancreas that was perfused with Krebs–Henseleit solution containing 2·5 mM glucose, 0·1% bovine serum albumin and 3% Dextran T-70 at a vascular flow rate of 1·2 ml min^{-1}.

2. Cholecystokinin-8 (CCK-8) at a concentration of 14 pM stimulated basal flow rate and amylase output of the isolated pancreas. Twenty-five millimolar glucose not only increased the basal flow rate and amylase output but also potentiated the CCK-stimulated flow rate and amylase output.

3. Porcine insulin, administered intra-arterially at a concentration of 100 nM, also increased the basal flow rate and amylase output, and also potentiated the CCK-stimulated flow rate and amylase output.

4. Rat PP, given intra-arterially at a concentration of 10 pM, completely abolished the potentiation effects of both the 25 mM glucose and the exogenous insulin on the CCK-stimulated flow rate and amylase output. Rat PP also inhibited the flow rate and amylase output increased by either 25 mM glucose alone or exogenous insulin alone. However, rat PP did not change the flow rate and amylase output stimulated by CCK-8 alone.

5. These results indicate that insulin is an important stimulatory hormone of pancreatic exocrine secretion, and that PP exerts the inhibitory role in pancreatic exocrine secretion by modulating the insulin action.

INTRODUCTION

Pancreatic polypeptide (PP) has been known to exert an inhibitory role in pancreatic exocrine secretion. PP infused intravenously inhibits pancreatic exocrine secretion stimulated by secretin and/or cholecystokinin (CCK) in humans (Adrian, Besterman, Mallinson, Greenberg & Bloom, 1978; Greenberg, McCloy, Adrian, Chadwick, Baron & Bloom, 1978), dogs (Lin, Evans, Chance & Spray, 1977) and rats (Louie, Williams & Owyang, 1985). It also inhibits pancreatic exocrine secretion stimulated by food in dogs (Schwartz, 1983; Lee, Shiratori, Chen, Chang & Chey, 1986; Shiratori, Lee, Chang, Jo, Coy & Chey, 1988). Furthermore, infusion of PP antibody results in a marked increase in pancreatic exocrine secretion during the interdigestive and digestive period in dogs (Shiratori *et al.* 1988). It has been

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reported, however, that PP does not inhibit CCK-stimulated amylase secretion from the incubated pancreas or dispersed acini of rats (Kim & Case, 1980; Louie, Williams & Owyang, 1985) and the perfused pancreas of cats (Kim & Case, 1980). Thus, the inhibitory mechanism of PP is still unclear. An indirect mechanism for the inhibitory action of PP on pancreatic exocrine secretion has been suggested since radiolabelled PP does not bind to the rat acini (Louie *et al.* 1985).

Insulin has been known to exert a very important role in pancreatic exocrine secretion particularly that stimulated by gut hormones and food (Kanno & Saito, 1976; Saito, Williams & Kanno, 1980; Singh, 1985; Williams & Goldfine, 1985; Lee, Zhou, Ren, Chang & Chey, 1990). There are some reports suggesting a possible interaction of PP and insulin. It has been reported that the plasma concentration of PP is elevated in patients (Floyd, Fajans & Pek, 1977; Service, Koch, Jay, Rizza & Go, 1985) and rats (Gingerich, Gersell, Greider, Einke & Lacy, 1978) with diabetes mellitus in which pancreatic exocrine secretion is impaired (Chey, Shay & Schuman, 1963; Williams & Goldfine, 1985). It has also been documented that the PP content in the pancreas increases in hyperglycaemic *ob,ob* mice (Gingerich, Gersell, Greider, Einke & Lacy, 1978) and that PP inhibits insulin release in genetically obese mice (Gettys, Garcia, Savage, Whitcomb, Kanayama & Taylor, 1991). However, the effects of PP on the insulin action in pancreatic exocrine secretion has not yet been determined.

The purpose of the present study, therefore, was to investigate a possible role of PP in the insulin action on CCK-stimulated pancreatic exocrine secretion using a totally isolated, vascularly perfused rat pancreas.

METHODS

Experimental animals

A total of sixty-eight male Sprague–Dawley rats, weighing 210–285 g, were anaesthetized with intraperitoneal injections of 25% urethane (Sigma, USA) at a dose of 0·7 ml (100 g of body weight)$^{-1}$. The rats were killed by intravenous overdose of urethane after the isolation of the pancreas. For the purpose of food control, rats were kept in this laboratory for at least 2 weeks before use. Food was withheld from rats, but they were allowed to drink water freely.

Isolation of the pancreas

An isolated rat pancreas was prepared according to a previously described method (Penhos, Wu, Basabe, Lopez & Wolff, 1969; Kanno & Saito, 1976) with minor modification. In brief, blood vessels supplying the stomach, liver and spleen were ligated and severed. Similarly, blood vessels between the pancreas and the adjacent tissue including the colon were carefully dissected and ligated. The abdominal aorta was carefully dissected and cannulated with PE-50 tubing (i.d. 0·58 mm, o.d. 0·97 mm; Clay Adams, USA) just above the coeliac artery, while the aorta below the mesenteric artery was tightly ligated. The portal vein was also cannulated with Tygon microbore tubing (i.d. 1·27 mm, o.d. 2·28 mm; Fisher Scientific, USA). After insertion of a plastic tube (i.d. 1·8 mm, o.d. 2·2 mm) into the proximal duodenum via the stomach, the pylorus was ligated and then the stomach was removed. The proximal jejunum was also cannulated with a plastic tube (i.d. 2·6 mm, o.d. 3·2 mm) near the ligament of Treitz and the intestine distal to the tube was removed. Thus, the inlet of vascular perfusion was the coeliac and mesenteric arteries, and the outlet was the portal vein. For pancreatic juice collection, PE-10 tubing (i.d. 0·28 mm, o.d. 0·61 mm; Clay Adams, USA) was inserted into the common bile duct near the duodenum and then the hepatic end of the common bile duct was ligated. Krebs–Henseleit solution (pH 7·4, 305 mosmol (kg water)$^{-1}$) containing 0·1% bovine serum albumin (Sigma, USA) and 3% Dextran T-70 (Sigma, USA) was perfused using a multistaltic pump (Buchler, USA) and the vascular flow rate was kept constant

at 1·2 ml min^{-1}. The medium was continuously oxygenated with 95% O_2, 5% CO_2. The isolated pancreas, including the duodenum, was placed in a temperature-controlled chamber (37 °C) which was continuously supplied with Krebs–Henseleit solution at a rate of 0·35 ml min^{-1} and the gas mixture. The isolated pancreas was perfused with medium containing 2·5 mM glucose during the equilibration period of 30 min and the basal period of 30 min. Samples of pancreatic juice were collected every 15 min from the isolated pancreas during the whole period of the experiment.

Effects of endogenous and exogenous insulin on pancreatic exocrine secretion

In the control experiment, 2·5 mM glucose medium was perfused for 45 min after the basal period and then sulphated CCK-8 (Squibb, USA) was added to the medium at a concentration of 14 pM. In order to see the effect of endogenous insulin on pancreatic exocrine secretion, 25 mM glucose was perfused for 45 min and then CCK-8 was added to the medium. The same protocol as above was repeated in the pancreas isolated from streptozotocin-treated rats to verify that 25 mM glucose affects the pancreatic exocrine secretion by endogenous release of insulin. Streptozotocin was intraperitoneally injected at a dose of 75 mg kg^{-1} 24 h before the experiment. For the purpose of observing the effect of exogenous insulin on pancreatic exocrine secretion, porcine insulin (Sigma, USA) at a concentration of 100 nM in 2·5 mM glucose medium was perfused for 45 min after which CCK-8 was added to the medium.

Effects of rat PP on insulin action

First of all, an effect of PP on the action of CCK alone in pancreatic exocrine secretion was observed. Rat PP (Peninsula, USA), at a concentration of 10 pM, in 2·5 mM glucose medium was perfused for 45 min and then CCK-8 was added to the medium. For the purpose of observing an effect of PP on the endogenous insulin action, rat PP in 25 mM glucose medium was perfused for 45 min and then CCK-8 was added to the medium. In order to see an effect of PP on the exogenous insulin action, rat PP together with porcine insulin in 2·5 mM glucose medium was perfused for 45 min after which CCK-8 was added to the medium.

Assay

Amylase activity in pancreatic juice was determined by the method of Rick & Stegbauer (1974).

Analysis of data

All data were expressed as means ± S.E.M. Student's *t* test or paired *t* test was used for statistical analysis of the data. *P* values less than 0·05 were considered significant.

RESULTS

Effects of endogenous and exogenous insulin on basal pancreatic secretion

As shown in Fig. 1, the flow rate and amylase output of the isolated pancreas during the basal period in which 2·5 mM glucose was perfused were $1·18 \pm 0·90$ μl $(15 \text{ min})^{-1}$ and $5·07 \pm 1·61$ U $(15 \text{ min})^{-1}$, respectively. When the glucose concentration in the perfusate was changed from 2·5 to 25 mM, the basal flow rate and amylase output were significantly ($P < 0·05$) elevated to $2·41 \pm 0·37$ μl $(15 \text{ min})^{-1}$ and $26·77 \pm 6·11$ U $(15 \text{ min})^{-1}$, respectively. However, the 25 mM glucose failed to elevate the basal flow rate and amylase output in the streptozotocin-treated pancreas (data not shown). Intra-arterial infusion of porcine insulin at a concentration of 100 nM also significantly ($P < 0·05$) increased the basal flow rate and amylase output to $2·67 \pm 0·40$ μl $(15 \text{ min})^{-1}$ and $14·68 \pm 2·82$ U $(15 \text{ min})^{-1}$, respectively.

Effects of endogenous and exogenous insulin on CCK-stimulated pancreatic secretion

Figure 1 also shows the effect of insulin on CCK-stimulated exocrine secretion of the isolated rat pancreas. CCK-8, given intra-arterially at a concentration of 14 pM in 2·5 mM glucose, significantly ($P < 0·01$) increased the basal flow rate and amylase

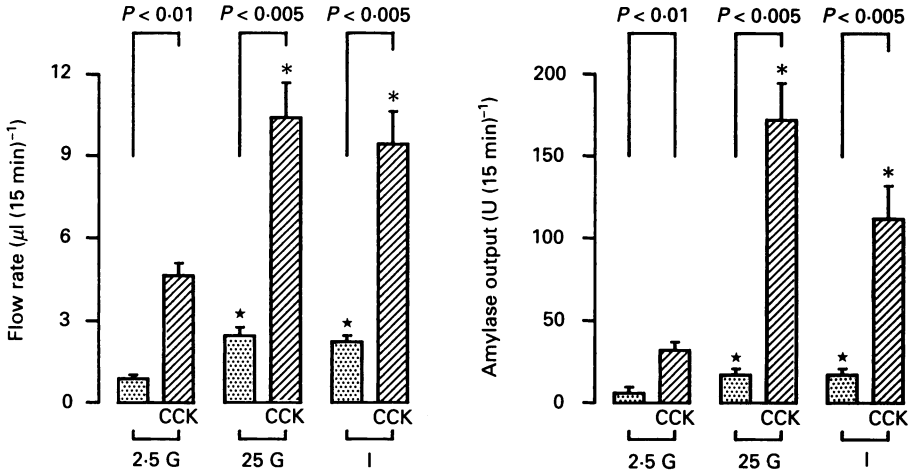

Fig. 1. Effects of endogenous (25 G) and exogenous (I) insulin on the basal secretion (▨) and CCK-8 (14 pM; ▨)-stimulated secretion of the isolated rat pancreas. For details see Figs 2 and 3. ★ ($P < 0.05$) or * ($P < 0.01$) indicate that the value is significantly different from the corresponding value at 2·5 G. Values are presented as means ± S.E.M. of pancreatic flow rate (left panel) and amylase output (right panel).

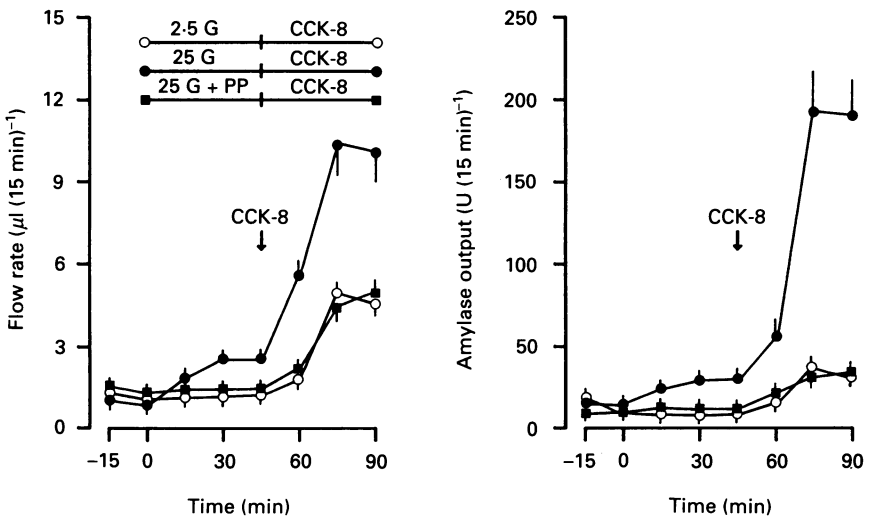

Fig. 2. Effect of rat PP on endogenous insulin action in CCK-stimulated secretion of the totally isolated, vascularly perfused rat pancreas. All pancreata were equilibrated with media containing 2·5 mM glucose (2·5 G) during the basal period. Release of endogenous insulin was induced by perfusion of 25 mM glucose (25 G). The medium containing 25 G was perfused in the presence (■) or absence (●) of rat PP (10 pM) for 45 min and then CCK-8 (14 pM) was added to the medium. In the control experiment (○), 2·5 mM glucose (2·5 G) was perfused for 45 min and then CCK-8 was added to the medium. Values are presented as means ± S.E.M. of pancreatic flow rate (left panel) and amylase output (right panel) obtained from eight pancreata (○), ten pancreata (●) and eight pancreata (■).

output to $4{\cdot}77 \pm 0{\cdot}42$ μl $(15\ \text{min})^{-1}$ and $25{\cdot}43 \pm 5{\cdot}64$ U $(15\ \text{min})^{-1}$, respectively. When 25 mM glucose was infused for 45 min and then CCK-8 was added, the pancreatic flow rate and amylase output were further elevated to $10{\cdot}27 \pm 1{\cdot}30$ μl $(15\ \text{min})^{-1}$ and

178·14 ± 39·94 U (15 min)$^{-1}$, respectively. Thus, potentiation occurred. However, the 25 mM glucose failed to potentiate the CCK-stimulated flow rate and amylase output in the streptozotocin-treated pancreas (data not shown). Intra-arterial infusion of porcine insulin at a concentration of 100 nM also potentiated the CCK-stimulated

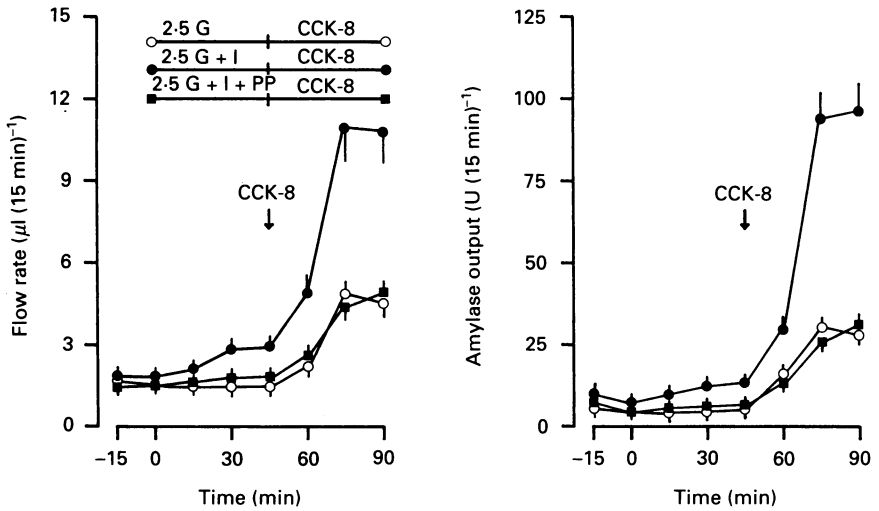

Fig. 3. Effect of rat PP on exogenous insulin (I) action in CCK-stimulated exocrine secretion of the isolated rat pancreas. All pancreata were equilibrated during the basal period with the medium containing 2·5 mM glucose (2·5 G). Porcine insulin (100 nM) in the presence (■) or absence (●) of rat PP (10 pM) in the 2·5 mM glucose medium was administered for 45 min and then CCK-8 (14 pM) was added to the medium. In the control experiment (○), 2·5 mM glucose (2·5 G) was perfused for 45 min and then CCK-8 was added to the medium. Values are presented as means ± S.E.M. of pancreatic flow rate (left panel) and amylase output (right panel) obtained from eight pancreata (○), eight pancreata (●) and nine pancreata (■).

flow rate and amylase output to 9·75 ± 1·02 μl (15 min)$^{-1}$ and 106·87 ± 23·13 U (15 min)$^{-1}$, respectively.

Effect of rat PP on insulin action

The effect of exogenous rate PP (10 pM) on the endogenous insulin action in the isolated pancreas is shown in Figs 2 and 4. When rat PP was administered intra-arterially, the flow rate and amylase output, which had been induced by 25 mM glucose alone, were reduced to the basal level (Fig. 2). PP also completely abolished the potentiation effect of 25 mM glucose on the CCK-stimulated flow rate and amylase output to 5·03 ± 0·91 μl (15 min)$^{-1}$ and 22·26 ± 3·90 U (15 min)$^{-1}$, respectively (Figs 2 and 4). The effect of PP on the exogenous insulin action in the isolated pancreas is shown in Figs 3 and 4. PP also diminished the flow rate and amylase output, which had been induced by exogenous insulin alone, back to the basal level (Fig. 3). PP also completely suppressed the potentiation effect of exogenous insulin on the CCK-stimulated flow rate and amylase output to 5·00 ± 0·56 μl (15 min)$^{-1}$ and 22·80 ± 3·90 U (15 min)$^{-1}$, respectively (Figs 3 and 4). However, as shown in Figs 4

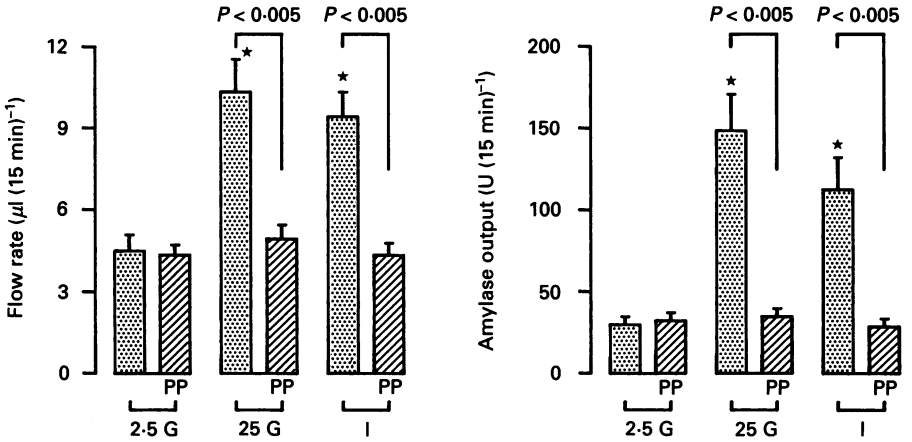

Fig. 4. Effect of PP on endogenous (25 G) and exogenous (I) insulin action in CCK-stimulated exocrine secretion of the isolated rat pancreas. For details see Figs 2, 3 and 5. ★ indicates that the value is significantly ($P < 0·005$) different from the corresponding value of CCK-8 (▨) only. Values are presented as means ± S.E.M. of pancreatic flow rate (left panel) and amylase output (right panel).

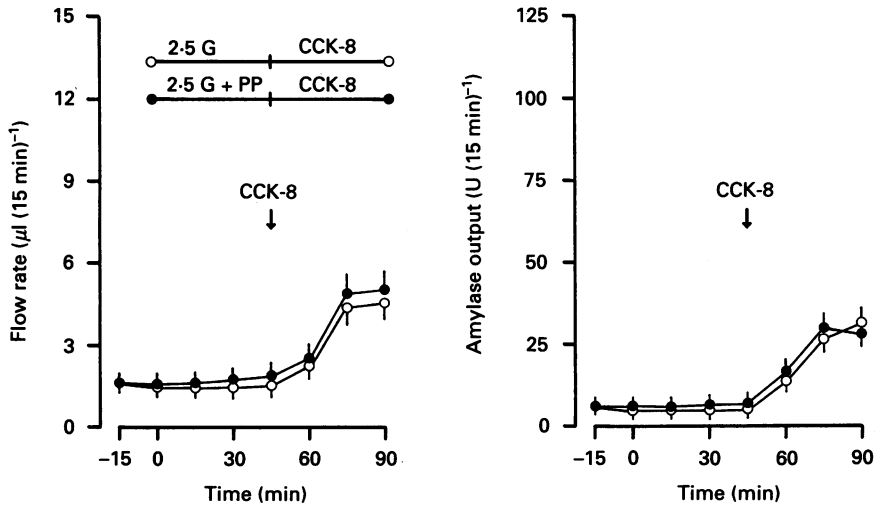

Fig. 5. Effect of rat PP on pancreatic exocrine secretion stimulated by CCK-8 alone. A glucose medium, 2·5 mM, in the presence (●, $n = 10$) or absence (○, $n = 8$) of PP (10 pM) was perfused for 45 min and then CCK-8 (14 pM) was added to the medium. Values are presented as a means ± S.E.M. of pancreatic flow rate (left panel) and amylase output (right panel).

and 5, PP failed to change the flow rate and amylase output stimulated by CCK-8 (14 pM) alone.

DISCUSSION

In the present study, both 25 mM glucose and exogenous insulin increased the basal secretion and potentiated CCK-stimulated secretion in the pancreas isolated from the normal rats. However, the effects of 25 mM glucose were not observed in the

pancreas isolated from the streptozotocin-treated rats. The results suggest that 25 mM glucose stimulates pancreatic exocrine secretion by induction of insulin release from the β-cells rather than direct action on the acinar cells. The results of this study, that endogenous and exogenous insulin potentiate CCK-stimulated pancreatic secretion, are in good agreement with those published previously (Kanno & Saito, 1976; Saito *et al.* 1980; Garry, Garry, Williams, Maloney & Sorenson, 1989) but in contrast to those of Bruzzone, Trimble, Ginovoci & Renold (1984), who reported that both glucose and exogenous insulin suppressed caerulein-stimulated amylase secretion. The reason for the discrepancy is not clear at the present time. In the cat (Wizemann, Weppler & Mahrt, 1974) exogenous insulin seems to have no effect on the pancreatic exocrine secretion stimulated by CCK. This may be due to species differences but more careful study is needed to elucidate species differences in insulin action on the pancreatic exocrine secretion. Nevertheless, evidence that insulin is of importance in the stimulation of pancreatic exocrine secretion in rats is increasing. It has been well documented that insulin stimulates protein synthesis (Korc, Iwamoto, Sankaran, Williams & Goldfine, 1981) and regulates amylase synthesis with its mRNA level (Korc *et al.* 1981; Duan, Poensgen, Wicker, Westrom & Erlanson-Albertsson, 1989). Recently, anti-insulin sera have been reported to completely block the pancreatic exocrine secretion stimulated by food or secretin and CCK-8 in conscious rats (Lee *et al.* 1990).

The most important finding of the present investigation is that exogenous rat PP abolished the insulin action in the isolated rat pancreas. PP inhibited pancreatic exocrine secretion stimulated by 25 mM glucose alone. Furthermore, PP completely eliminated the potentiation action of 25 mM glucose on the CCK-stimulated pancreatic secretion. From the above results it is not clear whether PP inhibits insulin action or reduces insulin release since high doses of PP (more than 50 $\mu g\ kg^{-1}$) have been reported to reduce insulin release in conscious rats (Gettys *et al.* 1991). However, a very low dose of PP (10 pM) was used in this study. Moreover, in this study, PP also inhibited pancreatic exocrine secretion stimulated by exogenous insulin alone and completely blocked the potentiation action of exogenous insulin on the CCK-stimulated pancreatic secretion. Therefore, we suspect that PP inhibits the pancreatic exocrine secretion by blocking insulin action rather than insulin release. It does not appear to be true that CCK action in the pancreas is modulated by PP. Although the results reported in a paper by Raymond & DeJoseph (1986) suggest otherwise, PP did not alter pancreatic exocrine secretion stimulated by CCK alone in the present study. PP also failed to produce change in amylase secretion induced by CCK alone in the dispersed rat pancreatic lobule (Louie *et al.* 1985) and incubated rat pancreas (Kim & Case, 1980) as well as in the isolated cat pancreas (Kim & Case, 1980). It remains uncertain at the present time how PP is able to interfere in the insulin action in the pancreas since binding of isotopically labelled PP to the acinar cells was not observed (Louie *et al.* 1985).

Possible mechanisms of PP action in the exocrine pancreas have been suggested. Jung, Louie & Owyang (1987) proposed that PP inhibited pancreatic enzyme secretion by presynaptic modulation of acetylcholine release while Pan, Lu, Quan & Xue (1987) suggested that PP exerted the inhibitory action by competitive binding to muscarinic receptors. However, DeMar, Taylor & Fink (1991) observed that

PP persistently inhibited secretin and CCK-stimulated pancreatic secretion in vagotomized and atropinized dogs. Therefore, the hypothesis that PP modulates the cholinergic transmission in the pancreas is still controversial at the present time.

The results of the present study provide physiological evidence for the presence of the insulo-acinar portal system in rats (Lifson, Lassa & Dixit, 1985). Since a single-pass perfusion system was used in this study, insulin released from the islet must act on the acinar cells via the insulo-acinar portal system rather than other pathways. It should be elucidated in a future study if endogenous PP, which is released from the F cells in the islet, also acts on the acinar cells through the insulo-acinar portal system.

In conclusion, it can be stated that insulin increases the basal pancreatic exocrine secretion and potentiates CCK action. PP abolishes the potentiation effect of insulin on CCK-stimulated pancreatic exocrine secretion. It can also be concluded that PP inhibits pancreatic exocrine secretion induced by insulin alone but it does not affect pancreatic exocrine secretion stimulated by CCK alone. These results indicate that insulin is an important stimulator of pancreatic exocrine secretion in both the basal and the CCK-stimulated states, and that PP exerts an inhibitory role in pancreatic exocrine secretion by modulating the insulin action.

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