Differences in Stimulatory Effects between Rat Pancreatic Secretory Trypsin Inhibitor-61 and -56 on Rat Pancreas

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Abstract Two types of pancreatic secretory trypsin inhibitors (PSTIs) were recently purified from rat pancreatic juice. One consisted of 61 (PSTI-61) and the other of 56 (PSTI-56) amino acid residues. PSTI-61 has been reported to elicit cholecystokinin (CCK) release when injected into the duodenum. Since no information has been available about the action of PSTI-56 on CCK release, the two PSTIs were compared for their stimulatory effect on CCK release and pancreatic exocrine secretions in conscious rats after intraduodenal administration. Rats were prepared with bile and pancreatic fistulae and with two duodenal cannulae. Pancreatic juice was excluded from the duodenum for 48 h prior to the experiment because rat PSTIs were trypsin sensitive. PSTI-61 significantly stimulated pancreatic secretions and increased plasma CCK concentrations from 3.6 to 6.5 pm, whereas PSTI-56 had no effect on either CCK release or pancreatic secretions. It is suggested that the action as a regulator for CCK release and pancreatic secretions is possessed only by PSTI-61, but not by PSTI-56.

Key words: rat PSTI, exocrine secretion, pancreas, cholecystokinin, conscious rat.

It has been known that these exist two types of trypsin inhibitors, basic pancreatic trypsin inhibitor (BPTI; Kunitz-type inhibitor) and pancreatic secretory trypsin inhibitor (PSTI; Kazal-type inhibitor), in the pancreas (KUNITZ and NORTHROP, 1936; KAZAL et al., 1948). The role of BPTI remains unknown. PSTI
Monitor
peptide =
Rat PSTI-61 Gly-Asn-Pro-Pro-Ala-Glu-Val-Asn-Gly-Lys-Thr-Pro-
Rat PSTI-56 Lys-Val-Ile-Gly-Lys-Lys-Ala-
Human PSTI(-56) Asp-Ser-Leu-Gly-Arg-Glu-Ala-
Asn-Cys-Pro-Lys-Gln-Ile-Met-Gly-Cys-Pro-Arg-Ile-Tyr-Asp-Pro-
Asn-Cys-Pro-Asn-Thr-Leu-Val-Gly-Cys-Pro-Arg-Asp-Tyr-Asp-Pro-
Lys-Cys-Tyr-Asn-Glu-Leu-Asn-Gly-Cys-Pro-Arg-Asp-Tyr-Asp-Pro-
Val-Cys-Gly-Thr-Asn-Gly-Ile-Thr-Tyr-Pro-Ser-Glu-Cys-Ser-Leu-
Val-Cys-Gly-Thr-Asp-Gly-Lys-Thr-Tyr-Ala-asn-Glu-Cys-Ile-Leu-
Val-Cys-Gly-Thr-Asp-Gly-Asn-Thr-Tyr-Pro-Asn-Glu-Cys-Val-Leu-
Cys-Phe-Glu-Asn-Arg-Lys-Phe-Gly-Thr-Ser-Ile-His-Ile-Gln-Arg-
Cys-Phe-Glu-Asn-Arg-Lys-Phe-Gly-Thr-Ser-Ile-Arg-Ile-Gln-Arg-
Cys-Phe-Glu-Asn-Arg-Lys-Arg-Gln-Thr-Ser-Ile-Leu-Ile-Gln-Lys-
Arg-Gly-Thr-Cys
Arg-Gly-Leu-Cys
Ser-Gly-Pro-Cys

Fig. 1. Amino acid sequences of PSTI.

is secreted into the pancreatic juice and PSTI has been believed to prevent the premature activation of pancreatic enzyme by trypsin in the pancreas since KAZAL et al. (1948) first isolate PSTI from bovine pancreas. The structures of bovine (GREENE and BERTELT, 1969), porcine (BERTELT and GREENE, 1971), sheep (HOCHESTRASSER et al., 1969), and human (KIKUCHI et al., 1985) PSTIs were elucidated. Recent studies in humans indicate that the increase in plasma RSTI (56 amino acid residues) levels may reflect the response to severe injury, that is, PSTI may act as an acute-phase reactant (MATSUDA et al., 1985; OGAWA et al., 1985). In rats, there exist two species of PSTIs consisting of 61 and 56 amino acid residues, which appeared to be translation products of different genes, while other animal species have only one PSTI (UDA et al., 1988). Thus, it has been suggested that rat PSTIs may possess a function(s) other than inhibition of trypsin activity. IWAI et al. (1987, 1989) reported that the peptide purified from rat pancreatic juice (called monitor peptide) stimulated cholecystokinin (CCK) release in anesthesized rats. This peptide also consisted of 61 amino acids and was considered to be identical to PSTI-61 (IWAI et al., 1989). On the other hand, the amino acid sequence of PSTI-56 has 29% sequence variation compared to PSTI-61, sharing 35 out of 56 amino acids with human PSTI while PSTI-61 shares 31 out of 61 amino acid residues (Fig. 1). We have recently reported that human PSTI had no stimulatory effect on rat pancreas (MIYASAKA et al., 1989). In the present study, we compared the effect of two PSTIs on CCK release and pancreatic exocrine secretion in conscious rats.
MATERIALS AND METHODS

Synthetic CCK-octapeptide sulfate (CCK-8) was purchased from Peptide Institute, Inc. (Osaka), taurocholic acid from Difco Laboratories (Detroit, Michigan, U.S.A.); bovine serum albumin (BSA), soybean trypsin inhibitor (type I-S), chromatographically purified collagenase (type IV) were from Sigma Chemical (St. Louis, MO, U.S.A.). Minimal Eagle's medium amino acid supplement was from GIBCO Laboratories, Life Technologies, Inc. (Ohio, U.S.A.), HEPES from Calbiochem-Behring (La Jolla, Ca, U.S.A.). Rat PSTIs were extracted and purified by HPLC from rat pure pancreatic juice according to the method previously reported (UDA et al., 1988).

Animal preparations. Male Wistar rats (314–330 g) and female Sprague-Dawley rats (200–259 g) were obtained from Shizuoka Jikken Dobutsu (Shizuoka, Japan). Rats were fed commercial rat chow (CRF 1, Oriental, Tokyo) before surgery and during recovery. The operative procedures were described in detail elsewhere (MIYASAKA and GREEN, 1984; MIYASAKA et al., 1986). Briefly, a midline abdominal incision was made under enflurane anesthesia (Abbott, North Chicago, IL, U.S.A.) delivered through a plastic face mask by means of a vaporizer. A cannula (Silastic Medical Grade Tubing, Dow Corning, Midland, MI, U.S.A.; 0.025 in. inside diameter x 0.037 in. outside diameter) was inserted into the common bile duct proximal to the ampulla of Vater. Then the common bile duct was ligated proximal to the pancreas near the liver, and the second cannula was inserted above the ligation below the bifurcation of the bile duct. Thus, pure bile and pure pancreatic juice were separately collected. Two additional cannulae were inserted into the duodenum, one for returning bile and pancreatic juice (BPJ), the other for PSTI injection. Their outlet tips were located near the ampulla of Vater. All cannulae were initially brought into the abdominal cavity through a subcutaneous channel starting at the back near the tail. The last cannula was inserted into the right jugular vein. After the operation, rats were placed in modified Bollman-type restraint cages. Animals were maintained in a 24°C room with filtered air, and light was scheduled from 05:00 through 17:00. BPJ was continuously returned via a duodenal cannula for 24 h after the surgery by a servo system consisting of a collection tube in a liquid level photo detector coupled to a peristaltic pump. Then, the returning of pancreatic juice was stopped to eliminate luminal trypsin activity for the following 48 h during which bile was continuously returned to the intestine, because PSTIs were sensitive to trypsic digestion. Experiments were conducted on the third postoperative day after 5 h of fasting because at least three days were required for the complete recovery of pancreatic exocrine function from the operative procedure (MIYASAKA et al., 1986).

Experimental protocols. The infusion of 40 mM taurocholate solution was started instead of the bile from the beginning of the experiment at a rate of 1 ml/h by a syringe pump (Harvard Apparatus Compact Infusion Pump, Harvard
Apparatus, Southnatick, MA, U.S.A.) because the exclusion of bile from the intestine has been known to produce pancreatic hypersecretion (Green and Nasset 1977, 1980). The intraduodenal infusion of taurocholate during bile diversion could prevent the hypersecretion (Nakamura et al., 1989). Pancreatic juice was collected at 15-min intervals. After 2 h of pancreatic juice collection, 1 ml of PSTI-61 (2 µg/ml) or PSTI-56 (2 or 10 µg/ml) was injected into the duodenum over the period of 1 min. PSTIs were diluted with isotonic saline. Pancreatic juice was collected for the following 15 min and 5 ml of blood was taken from the jugular vein cannula at the end of experiment. Isotonic saline (1 ml) was injected as a control. Blood samples were centrifuged at 3,000 rpm for 15 min at 4°C, and the plasma samples were kept at −70°C until they were assayed.

Assays. The volumes of bile and pancreatic juice were measured by a Hamilton syringe. Protein in pancreatic juice was estimated by determining optical density at 280 nm (Keller et al., 1958) of samples diluted 200 times in 0.04 M Tris buffer, pH 7.8. Bicarbonate concentration was measured by Natelson microgasometer using a 10 µl sample immediately after each collection. Plasma CCK level was measured by a bioassay using dispersed pancreatic acini (Liddle et al., 1984). The acini, obtained by collagenase digestion (Williams et al., 1978) from ovariectomized female Sprague-Dawley rats were incubated in polyethylene vials containing aliquots of acini suspension (Liddle et al., 1984; Louie et al., 1986). Amylase release into the medium and total acinar amylase contents were measured by using the blue starch polymer (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan, Neo-Amylase Test, in co-operation with the Pharmacia Diagnostic AB, Sweden) as the substrate. Values were compared with a standard curve of CCK-8 and results were expressed as CCK-8 equivalents.

Analysis of DATA. Values are expressed as the mean ± S.E. Results were analyzed by multiple analysis of variance (MANOVA) with repeated measures for changes of pancreatic secretions followed by Duncan's multiple range test and by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test for CCK concentration (Wallenstein et al., 1980). A value of \( p < 0.05 \) was considered significant.

RESULTS

Effect of rat PSTIs on pancreatic exocrine secretion

Pancreatic secretions were constant during the 2-h experimental period before the PSTI injection; therefore, only the values during the 30-min collection periods before the injection and the 15-min sample value immediately after the injection are illustrated in the figure. Two µg of PSTI-61 increased pancreatic juice flow, bicarbonate and protein outputs (Fig. 2). Neither of the two PSTI-56 doses (2, 10 µg) nor saline significantly affected pancreatic secretions. Statistical analysis by means of MANOVA revealed that there were significant differences among animals given different treatments in fluid, bicarbonate and protein outputs (\( F(3,34) = 7.30 \) for...
fluid, 5.07 for bicarbonate, 9.21 for protein output, p < 0.001). The fluid and protein outputs stimulated by 2 µg of PSTI-61 were significantly higher than the corresponding values stimulated by 2 µg of PSTI-56 and saline by multiple range test, whereas bicarbonate output was not significantly different from the latter two values. However, these values stimulated by PSTI-61 were not statistically higher than the respective values produced by 10 µg of PSTI-56 as evaluated by

Fig. 2. Pancreatic exocrine responses to PSTI. Upper panel shows the pancreatic responses to 2 µg of PSTI-61 (○) and saline (●), and lower panel, to 10 µg of PSTI-56 (●) and 2 µg of PSTI-56 (□). N = 7 for PSTI-61, 14 for 2 µg of PSTI-56, 10 for 10 µg of PSTI-56, and 7 for saline. Values with asterisks indicate a significant increase in comparison with the corresponding values produced by 2 µg of PSTI-56 or saline by multiple range test.

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Effect of PSTIs on plasma CCK concentrations

The injection of PSTI-61 produced a significant increase in plasma CCK concentration (Fig. 3). Plasma CCK concentrations were significantly different among the four treatments. The value stimulated by PSTI-61 was significantly higher than the values by either 2 µg PSTI-56 or saline injection but the difference from the value produced by 10 µg PSTI-56, 7 for saline.

DISCUSSION

The present results indicate that one of the two rat PSTIs tested (PSTI-61) elicits CCK release and stimulates pancreatic secretions but the other (PSTI-56) does not. It has been reported that the monitor peptide which is identical to PSTI-61 stimulates CCK release and pancreatic secretion in atropinized and anesthesized rats (Iwai et al., 1987, 1989). We furthermore confirmed the stimulatory effect of the monitor peptide in conscious rats (MIYASAKA et al., 1989). In the previous study, we diverted pancreatic juice from the intestine for 2 h before the monitor peptide injection because monitor peptide was trypsin sensitive (Iwai et al., 1987, 1989). That is, monitor peptide (PSTI-61) cannot elicit its trypsin inhibitory activity when

Fig. 3. Plasma CCK concentrations in response to PSTI administration. PSTI-61 significantly increased plasma CCK concentration as evaluated by ANOVA. The value with asterisk was significantly higher than values produced by 2 µg of PSTI-56 and saline injection. N = 7 for PSTI-61, 8 for 2 µg of PSTI-56, 7 for 10 µg PSTI-56, 7 for saline.
infused into the intestine under the condition of normal bile and pancreatic flow because it is immediately digested in the lumen. Furthermore, the inhibitory activity is known to be weak and temporary (Laszkowski and Wu, 1953). However, since it was not certain whether luminal protease activities could be completely eliminated by the 2-h pancreatic juice diversion, we simultaneously infused aprotinin in the duodenum. In the present study, in order to sufficiently decrease luminal trypsin, we diverted pancreatic juice from the intestine for 48 h before experiments. Since under normal bile flow, 5-h pancreatic juice diversion resulted in 98% decrease of trypsin activities in the proximal small intestine (Miyasaka and Green 1984), we could expect the sufficient decrease of luminal trypsin by this treatment. We confirmed again that PSTI-61 stimulated pancreatic secretions and CCK release in this model. However, PSTI-56 did not show any stimulatory effect. The mechanism of CCK release by PSTI-61 has not been clarified, and it is not clear whether it directly affects CCK-secreting cells or requires some mediators. It has been well known that intraduodenal injection of trypsin inhibitors stimulates CCK release and pancreatic secretion by means of a decrease in luminal protease activity (Green and Lyman, 1972; Louie et al., 1986). However, the inhibitory potency for trypsin was comparable for PSTI-61 and -56 (Uda et al., 1988). Furthermore, in this study, trypsin originating from pancreas was excluded by the external pancreatic fistula for 48 h before experiments. Therefore, it could be concluded that the stimulatory effect of PSTI-61 was not attributed to its trypsin inhibitory activity and that the differences in the effect on CCK release between the two were independent of their trypsin inhibitory activity. The observation that the potency of trypsin inhibitor’s CCK-releasing activity was independent of its potency of trypsin inhibitory activity is compatible with the observation of Yonezawa et al. (1984).

PSTI-56 is known to have a greater sequence homology with human PSTI than PSTI-61 (Uda et al., 1988). We have observed that larger doses of human PSTI (200 µg) and PSTI-56 (10 µg) slightly increased the CCK release and pancreatic secretions although the difference was not statistically significant (Miyasaka et al., 1989) (Figs. 2 and 3). Since we did not measure the trypsin inhibitory activity in the intestinal lumen after PSTI injections, we could not exclude the possibility that PSTI-56 might be more sensitive to tryptic digestion than PSTI-61 and more easily inactivated by residual trypsin in the intestine. These results raise the possibility that the stimulatory action of PSTI-61 on CCK release may be due to a certain molecular structure specific to PSTI-61 which is different from that of PSTI-56 or human PSTI.

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