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Research Article

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Feedback Regulation of Pancreatic Enzyme Secretion

Suppression of Cholecystokinin Release by Trypsin

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Abstract

Feedback regulation of pancreatic enzyme secretion occurs in rats. Whether such a system exists in man remains unsettled and the responsible mechanism is unknown. To investigate this question gastrointestinal intubation and perfusion were performed in 12 healthy subjects. Intraduodenal perfusion of trypsin-inhibited phenylalanine-, oleic acid-, and meal-stimulated chymotrypsin and lipase outputs in a dose-related manner. The minimal concentration of bovine trypsin needed to inhibit pancreatic enzyme secretion was 0.5 g/liter. 1 g/liter caused a maximal suppression of 35±4% of the phenylalanine-stimulated chymotrypsin release. This inhibitory effect was protease-specific. Intraduodenal perfusion of phenylalanine and oleic acid increased plasma cholecystokinin (CCK) from a basal level of 0.9±0.06 to 5.3±0.9 pM and 7.2±1.3 pM, respectively. Addition of bovine trypsin to the perfusates significantly reduced the plasma CCK level to basal values. This inhibitory effect of trypsin on CCK release was dose dependent and specific to proteases. Therefore, the present studies indicate that feedback regulation of pancreatic enzyme secretion is operative in man and it is mediated by release of CCK.

Introduction

Feedback inhibition of pancreatic enzyme secretion by pancreatic proteases in the duodenum has been demonstrated in a number of animals. Green and Lyman (1) demonstrated that diversion of pancreatico-biliary juice from the proximal intestine caused a marked increase in pancreatic enzyme secretion. Trypsin, chymotrypsin, or pancreatico-biliary juice infused into the duodenum suppressed pancreatic enzyme secretion.

Although a similar feedback control system has been found in the chicken (2, 3) and pig (4), such a regulatory control is not present in the dog (5). The question as to whether or not such a system exists in man remains unsettled. Ihse et al. (6) reported that when bile and pancreatic flow from the duodenum was completely obstructed by a carcinoma of the ampulla of Vater, infusion of active pancreatico-biliary juice or trypsin into the duodenum caused a rapid fall in pancreatic secretion, as measured by a cannula placed in the common bile duct. Conversely, intraduodenal infusion of pancreatic juice treated with soybean trypsin inhibitor (SBTI)[1] failed to suppress pancreatic secretion. These observations strongly suggest that feedback regulation of pancreatic enzyme secretion occurs in man. However, Krawicz et al. (7), using an intubation double-marker perfusion technique, demonstrated that diversion or reinfusion of active pancreatico-biliary secretion in the jejunum did not have any significant effect on saline-stimulated pancreatic enzyme secretion. They concluded that in the absence of nutrients, bile-pancreatic juice in the jejunum does not exert feedback control of human pancreatic secretion. Unfortunately their studies are not comparable to the rat or the human studies described above, since the technique used did not completely divert pancreatic juice from the duodenum. Therefore, further studies are needed to clarify this issue.

In this study we investigated the effects of intraduodenal perfusion of trypsin on basal as well as on phenylalanine-, oleic acid-, and meal-stimulated pancreatic enzyme secretion in man, and examined the role of cholecystokinin (CCK) in the negative feedback regulation of pancreatic enzyme secretion.

Methods

Materials. The following were purchased: SBTI (type I-S); trypsin (type III-S); purified porcine lipase and amylase; atropine sulfate; Tris (hydroxymethyl) aminomethane; P-tosyl-L-arginine methylester HCl; sulphated gastrin; N^2,O^2-dibutyryl guanosine 3',5' cyclic monophosphate (Bt_2cGMP); and phenylalanine from Sigma Chemical Co., St. Louis, MO. Chromatographically purified collagenase was purchased from Cooper Biomedical Inc., Malvern, PA; minimal Eagle's medium amino acid supplement from Gibco, Grand Island, NY; Hepes from Calbiochem-Behring Corp., LaJolla, CA; and bovine serum albumin (fraction V) from Miles Laboratories, Elkhart, IN. Cholecystokinin-octapeptide (CCK8), nonsulfated gastrin, and vasoactive intestinal peptide (VIP) were obtained from Peninsula Laboratories, Inc., Belmont, CA; SEP-PAK C-18 cartridges from Water Associates, Milford, MA; polyethylene glycol (PEG-4000) from Fisher Scientific, Fairlawn, NJ; Affi-Gel 10 and protein assay dye regent from Bio-Rad Laboratories, Richmond, CA; and procion yellow dye from Polysciences, Inc., Warrinton, PA. Lipomul was obtained from Upjohn Company, Kalamazoo, MI; oleic acid from Mallinckrodt, Inc., Paris, KY. CCK8 antiserum was a gift from Dr. Tadataka Yamada, University of Michigan, Ann Arbor, MI. Purified porcine CCK33 (Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm, Sweden) was a gift from Dr. John Williams, University of California, San Francisco, CA.

Subjects. Twelve healthy male volunteers, 19–34-yr-old, participated in the studies. All subjects were within 10% of their ideal body weight; none were taking any medication, or had any history of gastrointestinal symptoms and surgery. The studies were approved by the University of Michigan Human Use Committee on February 12, 1983, and written informed consent was obtained in each case.

This study was presented in part at the 84th Annual Meeting of the American Gastroenterological Association, Washington, DC, May 1983, and was published in abstract form, 1983. *Gastroenterology.* 84:1268.

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1. *Abbreviations used in this paper:* CCK, cholecystokinin; CCK8, cholecystokinin-octapeptide; Bt_2cGMP, N^2,O^2-dibutyryl guanosine 3',5' cyclic monophosphate; DF, degrees of freedom; PEG-4000, polyethylene glycol; SBTI, soybean trypsin inhibitor; TR, Tris (hydroxymethyl) aminomethane-buffered Ringer solution; VIP, vasoactive intestinal peptide.

Intraduodenal perfusion studies. Subjects were admitted to the University of Michigan Clinical Research Center and all studies were performed after an overnight fast. Pancreatic enzyme output was studied with the standard gastroduodenal intubation perfusion technique detailed elsewhere (8, 9). In brief, a double-lumen polyvinyl duodenal tube combined with a separate gastric sump tube were placed under fluoroscopic control, with the aspiration site at the ligament of Treitz and the perfusion site 20 cm proximal to the aspiration site in the second portion of the duodenum. The gastric sump tube was situated at the gastric antrum for continuous gastric aspiration. The average radiation exposure to the abdomen during fluoroscopy was 0.7 rad per study. Normal saline and test solutions were instilled into the second portion of the duodenum at 5 ml/min and the duodenal contents were recovered by constant suction (−20 mmHg) at the ligament of Treitz. These samples were collected into a flask immersed in ice and pooled at 15-min intervals.

We first investigated if basal trypsin secretion in the duodenum has an inhibitory effect on basal pancreatic secretion. SBTI was used to inactivate proteolytic enzymes in the duodenum. In vitro studies in our laboratory showed that 1 mg of SBTI inactivated 0.07 U of trypsin. Previous studies demonstrated that trypsin outputs during intraduodenal perfusion of normal saline seldom exceeded 25 kilounits (kU)/h (9). Therefore, a perfusion rate of 380 mg of SBTI per hour, which will inactivate 26.4 kU/h, should be sufficient to neutralize the basal trypsin secretion. In 12 healthy subjects, the duodenum was perfused with normal saline containing PEG-4000. After the steady state was established, basal duodenal juice samples were collected from the ligament of Treitz and pooled every 15 min for 2 h. The duodenum was then perfused with normal saline containing SBTI (1 mg/ml) and collections were made for an additional 2 h. During the study duodenal and blood samples were obtained every 15 min during basal and test periods.

We also investigated the effect of intraduodenal perfusion of trypsin on basal pancreatic secretion. The experimental protocol was similar to that described above. Instead of SBTI, the duodenum was perfused with bovine trypsin (1 g/liter).

To determine if intraduodenal perfusion of trypsin inhibits phenylalanine-stimulated pancreatic secretion, eight healthy volunteers were studied. After intubation and a steady state had been established by intraduodenal perfusion of normal saline, the duodenum was perfused with 10 mM phenylalanine without or with the addition of bovine trypsin (1 g/liter) for 90 min. Duodenal and blood samples were collected as previously described.

The minimal dose of bovine trypsin needed to inhibit phenylalanine-stimulated pancreatic enzyme secretion was determined by varying the dose of bovine trypsin infused into the duodenum. The experimental protocol consisted of five 90-min periods of intraduodenal perfusion of phenylalanine (10 mM) without or with addition of different doses of bovine trypsin (0.25, 0.5, 1, 2 g/liter). Each study period was separated by 60 min of normal saline perfusion.

In separate studies we investigated the effects of intraduodenal perfusion of trypsin on pancreatic enzyme secretion stimulated by oleic acid. The duodenum was perfused with oleic acid (9 mM) alone or with addition of trypsin (1 g/liter) for 90 min.

To demonstrate that feedback regulation of pancreatic enzyme secretion is of physiological importance we investigated the effect of intraduodenal perfusion of trypsin on pancreatic enzyme secretion stimulated by a standard solid meal. The standard meal used in this study consisted of ground round beef (90 g uncooked weight), flavored with salt (0.1 g); white bread (20 g) with butter (13 g); vanilla ice cream (60 g) topped with chocolate syrup (40 g) and a glass of water (240 ml). The total calories were 553 and these were distributed as 40% carbohydrates, 40% fat, and 20% protein. Four healthy subjects participated in the meal studies. Each subject was studied on two different days after ingesting the mixed meal with or without simultaneous intraduodenal perfusion of trypsin (1 g/liter) at 5 ml/min. Pancreatic enzyme output was quantified as described previously (9). During the study duodenal and blood samples were obtained every 20 min during basal and test periods.

To evaluate enzyme specificity in the feedback regulation of pancreatic enzyme secretion we examined the effect of intraduodenal perfusion of lipase and amylase on pancreatic enzyme secretion stimulated by phenylalanine. The experimental design was similar to that described above. The duodenum was perfused with phenylalanine (10 mM) in the absence or presence of either lipase (4.5 g/liter) or amylase (26.7 g/liter) for 90 min.

Bioassay of plasma CCK. CCK was extracted from plasma using modifications of methods described by Liddle et al. (10, 11). Plasma was absorbed onto C-18 SEP-PAK cartridges, which were previously washed with 10 ml acetonitrile, 10 ml ethanol, followed with 20 ml of water. Up to 6 ml of plasma were applied to the cartridges, followed by washing with 20 ml water. CCK was eluted with 1 ml acetonitrile/water (1:1) into a polyethylene scintillation vial and dried in a 45°C water bath under a flow of nitrogen. To assess recovery of CCK from the cartridges, known quantities of CCK, dissolved in Tris (hydroxymethyl) aminomethane-buffered Ringer solution (TR), were added to TR-buffer or plasma from fasting subjects and the CCK extracted by the procedures outlined above. Addition of CCK8 and CCK33 in concentrations ranging from 10 to 100 fmol yielded recoveries of 87±6% and 84±5%, respectively.

Isolated rat pancreatic acini were prepared by enzymatic digestion of pancreases from fasted, ovariectomized Sprague-Dawley rats as previously described (12, 19). Briefly, tissue was incubated for 50 min at 37°C in Krebs-Henseleit bicarbonate buffer containing 0.1% purified collagenase and supplemented with Eagle's minimum amino acids and gassed with 95% O_2/5% CO_2. Tissue was broken up with mild shearing forces and acini were then resuspended in TR solution. TR was similar to Krebs-Henseleit bicarbonate buffer, but contained 40 mM Tris as buffer, 0.5% bovine serum albumin, and was gassed with 100% O_2.

1-ml aliquots of acini suspension were added to the vials containing the plasma extracts or known amounts of CCK8 and incubated for 30 min at 37°C. Amylase released into the medium and total acinar amylase content were measured, using procion yellow starch as substrate (13). Amylase release was expressed as a percentage of total acinar amylase content. The percent release by the plasma extracts was compared with the dose-response curve to CCK8 to calculate the plasma CCK expressed as CCK8 equivalents.

Our preparations of rat pancreatic acini exhibited a biphasic dose response to CCK8. The threshold dose was 1 pM and maximal stimulation was seen at 300 pM CCK8. Sulfated and nonsulfated gastrin-17 were ~1000- and 1500-fold less potent than CCK8 in stimulating amylase release.

Intraduodenal perfusion of phenylalanine resulted in a rise in plasma CCK. To verify that the secretagogue extracted from plasma was CCK, various procedures were performed and were confirmatory of the results previously reported by Liddle et al. (10, 11). In brief, (*a*) plasma extracts were incubated with increasing concentrations of Bt_2cGMP, a specific CCK receptor antagonist. Amylase release was inhibited in a dose-dependent manner. High doses of Bt_2cGMP (≥0.3 mM) completely suppressed all CCK activity. The pattern of inhibition by Bt_2cGMP was similar to its inhibition of CCK8-stimulated amylase release. (*b*) Serial dilution of plasma paralleled the dose-response pattern of CCK8-stimulated amylase release. (*c*) Plasma extracts were applied onto an affinity chromatography column linked with an antibody directed at the carboxyl terminal of CCK. Bioassay of the wash eluates for CCK activity showed no detectable levels of CCK. CCK activity was observed when plasma extracts were eluted under acidic conditions. To evaluate any amylase release resulting from stimulation by muscarinic substances in plasma, atropine was added to plasma extracts. No decrease in amylase release by plasma extracts was observed. To assess if secretin or VIP were present in plasma at levels which would potentiate the effects of CCK (14), a dose of VIP, which results in maximal potentiation (1 nM), was added to the CCK8 standards and to plasma extracts. The increase in the amylase released from plasma extracts was comparable to the increase seen when VIP was added to CCK8.

Measurement of luminal pancreatic enzymes. The concentration of trypsin, chymotrypsin, and lipase was measured in all duodenal juice samples. Trypsin, chymotrypsin, and lipase concentrations were determined by means of a titrimetric method using *p*-tosyl-L-arginine meth-

ylester HCl, *N*-acetyl-L-tyrosine ethyl ester, and fat emulsion (Lipomul) as substrates for the respective enzyme activities (15). PEG-4000 (16) concentrations were determined by previously described spectrophotometric methods. The outputs of enzymes were then expressed in kilounits per hour based on recovery in relation to PEG-4000 (8). Pancreatic outputs during the last four 15-min intervals of each control and test period were used in the statistical analysis.

Statistical analysis. The intergrated CCK response was calculated by a previously described method (17). All results were expressed as mean±SE. Statistical analysis was performed using analysis of variance with comparison among groups using the method of Dunn. Significance was set at the 5% level.

Results

Pancreatic secretion studies. The mean (±SE) basal trypsin, chymotrypsin, and lipase outputs were 7±2, 9±3, and 26±6 kU/h, respectively. Intraduodenal perfusion of SBTI (1 mg/ml) completely inactivated basal tryptic activity but had no significant effect on basal pancreatic chymotrypsin (10±2 kU/h) or lipase (24±7 kU/h) secretion. Intraduodenal perfusion of trypsin (1 g/liter) resulted in a trypsin output of 36±12 kU/h, but it did not affect basal chymotrypsin (13±6 kU/h) or lipase (29±4 kU/h) output.

Intraduodenal perfusion of phenylalanine (10 mM) produced a significant increase in mean trypsin and chymotrypsin outputs which were 30±5 and 39±6 kU/h, respectively. On a separate day, the duodenum was perfused with phenylalanine (10 mM) and trypsin (1 g/liter). The duodenal trypsin output was 54±9 kU/h. The mean increase in chymotrypsin output was smaller compared with the responses to phenylalanine alone at all time intervals (Fig. 1).

As shown in Fig. 2, in all eight studies the rise in chymotrypsin output during phenylalanine (10 mM) plus trypsin (1 g/liter) perfusion was smaller than the responses to phenylalanine alone. Overall, the mean chymotrypsin outputs during intraduodenal perfusion of phenylalanine plus trypsin was 65±5.7% of that observed during perfusion of phenylalanine alone. The lipase responses to stimulation by phenylalanine without and with trypsin were similar to the chymotrypsin responses (data not shown).

A dose-response curve was established to determine the minimal dose of bovine trypsin needed for inhibition of phenylalanine-stimulated pancreatic enzyme secretion. As shown in Fig. 3, the minimal concentration of bovine trypsin required to suppress pancreatic chymotrypsin secretion was 0.5 g/liter and maximal suppression was observed with 1 g/liter of trypsin. When these doses of trypsin were used, duodenal trypsin outputs were 27±9 and 50±10 kU/h, respectively.

In response to intraduodenal perfusion of oleic acid (9 mM) the duodenal chymotrypsin output averaged 47±5 kU/h. Addition of bovine trypsin (1 g/liter) to the perfusate partially inhibited the chymotrypsin response to stimulation by oleic acid. This resulted in a chymotrypsin output of 32±6 kU/h, which represented 68±3% of control output.

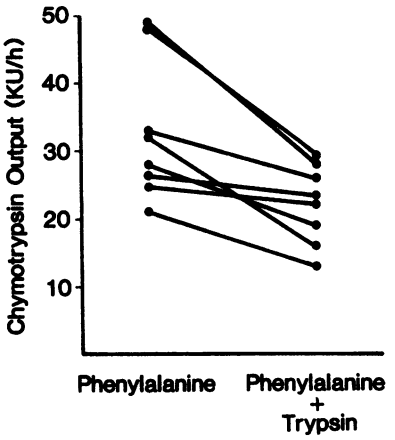

Figure 2. Influence of intraduodenal perfusion of trypsin on phenylalanine-stimulated chymotrypsin outputs. Effects are demonstrated by comparing chymotrypsin outputs during phenylalanine (control) and phenylalanine with bovine trypsin perfusion. Individual data for 8 subjects shown.

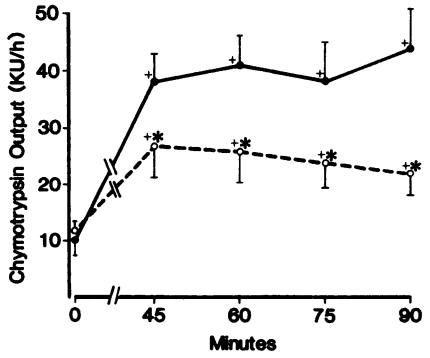

Figure 1. Mean (±SE) duodenal chymotrypsin output in response to intraduodenal perfusion of phenylalanine (10 mM) (— • —) and phenylalanine (10 mM) with bovine trypsin (1 g/liter). (--o--) Addition of trypsin produced a highly significant treatment effect (F = 39.074, DF 1/56, $P < 0.001$). Plus sign denotes points significantly different from basal. Asterisk denotes points significantly different from phenylalanine perfusion (n = 8). There was no effect of time (F = 0.499, DF, 3/56).

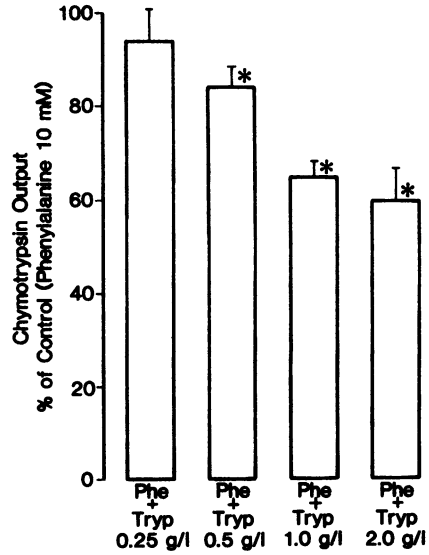

Figure 3. Effect of different doses of bovine trypsin on pancreatic chymotrypsin secretion stimulated by intraduodenal perfusion of phenylalanine (10 mM). Results are expressed as percent of chymotrypsin outputs in response to phenylalanine (10 mM) stimulation (n = 8). Trypsin produced a highly significant treatment effect (F = 90.591, DF, 3/28, $P < 0.001$). Asterisk denotes significantly different from control.

After ingestion of a test meal, chymotrypsin output increased rapidly, peaking within the first hour. The output then gradually declined, but still remained elevated above basal at 3 h postprandial (Fig. 4). On a separate day, when the duodenum was perfused with trypsin (1 g/liter), ingestion of the same test meal resulted in a significantly smaller increase in chymotrypsin outputs at all time intervals (Fig. 4).

In contrast to the inhibitory effect of trypsin on exocrine pancreatic secretion, the addition of lipase or amylase to the phenylalanine perfusate produced no significant change in chymotrypsin output (43±5 and 49±6 kU/h, respectively) compared with the outputs in response to intraduodenal perfusion of phenylalanine alone (37±6 kU/h).

Plasma CCK levels. After a 12-h fast, the basal plasma CCK levels were 0.9±0.06 pM (n = 12). The basal levels were not affected by intraduodenal perfusion of SBTI (1.1±0.08 pM) or trypsin (1.3±0.09 pM).

After intraduodenal perfusion of phenylalanine (10 mM) there was a prompt increase in plasma CCK levels to 5.3±0.9 pM within the first 15 min and the levels were maintained during the entire 90 min of perfusion (Fig. 5). In contrast, when the duodenum was perfused with the same dose of phenylalanine with bovine trypsin (1 g/liter), the mean plasma CCK levels were 1.7±0.5 pM. These were not significantly different from basal levels. The integrated plasma CCK responses over 90 min of intraduodenal perfusion of phenylalanine without and with bovine trypsin (1 g/liter), were 215±39 pM·min and 27±17 pM·min, respectively.

As shown in Fig. 6, bovine trypsin inhibited the plasma CCK response to phenylalanine stimulation in a dose-related manner. The minimal concentration of trypsin which affected the plasma CCK response was 0.5 g/liter. The optimal concentration of trypsin which showed maximal inhibition was 1 g/liter. Further increases in trypsin concentrations during phenylalanine stimulation did not decrease plasma CCK.

As with phenylalanine, intraduodenal perfusion of oleic acid stimulated plasma CCK levels to 7.2±1.3 pM. Addition of bovine trypsin (1 g/liter) to the perfusate significantly reduced the plasma CCK levels to 2.3±1.2 pM, which were not significantly different from basal.

After ingestion of the test meal there was a prompt increase in plasma CCK concentrations to 4.1±0.8 pM within 20 min, postprandial. The peak plasma CCK levels (7.3±1.2 pM) were reached at 60 min and were followed by gradual decline to 3.8±0.6 pM at 180 min after ingestion of the test meal. In contrast, when the duodenum was perfused with trypsin (1 g/liter), there was no postprandial increase in plasma CCK concentrations above basal (0.8±0.05 pM) during the entire study period of 180 min.

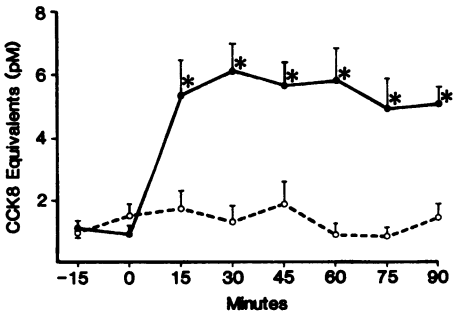

Figure 5. Plasma CCK response to intraduodenal perfusion of phenylalanine (10 mM) (— • —) and phenylalanine (10 mM) plus trypsin (1 g/liter) (--o--). Trypsin produced a highly significant treatment effect (F = 490.116, DF, 1/112, $P < 0.001$). Asterisk denotes significantly different from basal values. Values are the mean±SE CCK levels of eight subjects.

In contrast to the inhibitory effect of trypsin on CCK release, intraduodenal perfusion of lipase or amylase did not affect the plasma CCK response to phenylalanine (10 mM) stimulation (6.2±1.4 and 5.7±1.1 pM, respectively).

Discussion

In this study we have demonstrated that intraduodenal perfusion of trypsin inhibits phenylalanine-stimulated pancreatic enzyme secretion. This provides strong support that feedback regulation of pancreatic enzyme secretion operates in man. Our dose-response studies indicate that the minimal concentration of bovine trypsin required to exert inhibition on pancreatic enzyme secretion was 0.5 g/liter and maximal suppression was observed with 1 g/liter. These doses could be considered physiological since they produced trypsin outputs similar to those observed after a meal (18). Our studies also demonstrated that intraduodenal perfusion of trypsin inhibits meal-stimulated pancreatic enzyme secretion. The degree of inhibition was similar to that observed when phenylalanine was used as the stimulus. This further illustrates that the feedback regulation of pancreatic enzyme secretion is physiologically important.

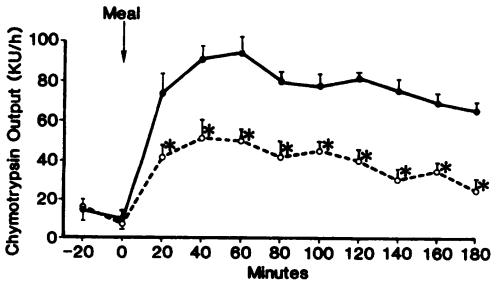

Figure 4. Mean (±SE) duodenal chymotrypsin outputs in response to a standard mixed meal without (— • —) and with (--o--) simultaneous intraduodenal perfusion of trypsin (1 g/liter). Trypsin significantly reduced meal-stimulated chymotrypsin outputs (F = 24.391, DF, 1/40, $P < 0.001$). Asterisk denotes points significantly different from control.

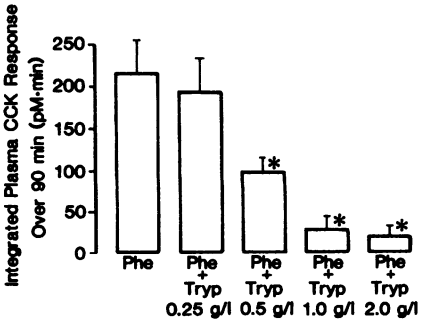

Figure 6. Integrated plasma CCK responses to intraduodenal perfusion of phenylalanine (10 mM) without and with addition of bovine trypsin. Trypsin produced a highly significant treatment effect (F = 109.168, DF, 4/35. $P < 0.001$). Asterisk denotes significantly different from control values (n = 8).

The suppression of pancreatic exocrine secretion appears to be enzyme-specific, since suppression was not observed with intraduodenal perfusion of lipase or amylase. Similar observations have been made in rat studies (19). In addition, Slaff et al. reported that intraduodenal perfusion with proteases, but not with amylase or lipase, suppresses pancreatic exocrine secretion in patients with chronic pancreatitis (20). This phenomenon appears to be dependent on the tryptic activity, since trypsin inhibitor negated the ability of trypsin to suppress pancreatic secretion (20). To rule out the possibility that the observed inhibition of pancreatic enzyme secretion after intraduodenal administration of trypsin is due to tryptic digestion of chymotrypsin, we have performed in vitro studies which indicated that addition of trypsin (1 g/liter) to known amounts of chymotrypsin did not affect chymotrypsin activity (Owyang, C., D. S. Louie, and D. Tatum, unpublished observation).

Although feedback regulation of pancreatic enzyme secretion appears to exist in man, there are some basic differences from the feedback regulation in rats, where administration of SBTI markedly stimulates the exocrine pancreas and trypsin in the duodenum inhibits basal pancreatic secretion (1). In our studies the mean basal trypsin output was 9±3 kU/h. Intraduodenal infusion of trypsin inhibitor completely inactivated the basal tryptic activity but had no significant effect on basal pancreatic chymotrypsin and lipase secretion. This suggests that in man the basal amounts of trypsin in the duodenum are too small to exert any inhibitory effect. This is not surprising, since the minimal amount of trypsin required to suppress pancreatic enzyme secretion is 27±9 kU/h. Furthermore, we also demonstrated that basal pancreatic secretion in man was not suppressible by intraduodenal trypsin. This may explain the findings of Krawicz et al., who reported that in the absence of nutrients, bile-pancreatic juice in the jejunum does not exert feedback control of human pancreatic secretion (7).

The mechanism responsible for the feedback regulation of pancreatic enzyme secretion is unknown. Several studies suggest that this is mediated by a hormone secreted by the proximal small intestine. When plasma from an animal fed SBTI was perfused into an isolated rat pancreas, amylase output was increased. The response was unaffected by addition of atropine (21). If the duodenum and jejunum in rats were resected, the increased enzyme secretion after administration of SBTI was abolished (22). Thus, both the location of release and the biological action of the substance released by SBTI are similar to those of CCK (23–25). This suggests that the most likely candidate for the hormone involved is CCK. Evaluation of the role of CCK in the feedback regulation of pancreatic enzyme secretion has been impeded by nonspecific and insensitive assays for CCK. Bioassays for CCK, based on gallbladder contractions (26), are too insensitive to detect plasma CCK levels, and radioimmunoassays for CCK lack specificity due to their cross-reactivity with gastrin. Our measurements of CCK are based on release of amylase from isolated rat pancreatic acini. The degree of specificity of this assay for CCK is founded on the difference in potency between CCK and gastrin in eliciting amylase release from acini. Sulfated and nonsulfated gastrin-17 are ~1000- and 1500-fold less potent than CCK8 in stimulating amylase release. If gastrin were present in the extracted plasma, abnormally high levels of gastrin must be present to account for a small fraction of the stimulated amylase release. In our studies basal gastrin levels in man as measured by previously reported radioimmunoassay (27) were 50–70 pM. Intraduodenal perfusion of phenylalanine or oleic acid did not change basal plasma gastrin levels significantly. Thus, this bioassay system allows us to measure plasma CCK levels without interference from gastrin.

Basal plasma CCK concentrations in man were low as measured by this bioassay. The values are similar to those reported by Liddle et al. (11). In man, similar low values estimated by radioimmunoassay have been reported (28). Significant increases in plasma CCK levels were observed after intraduodenal perfusion of phenylalanine, oleic acid, or after ingestion of a mixed meal. Liddle et al. (11) observed a similar rise in plasma CCK levels in response to oral administration of amino acids and fat. The nutrient-stimulated plasma CCK levels based on the bioassay system are similar to those estimated by radioimmunoassay (28). The threshold plasma levels of CCK needed to stimulate pancreatic secretion in man are in the range of 3–5 pM (29). Thus, the rise in plasma CCK concentration postprandially or after intraduodenal perfusion of phenylalanine and oleic acid should be sufficient to stimulate pancreatic enzyme secretion. Intraduodenal perfusion of bovine trypsin suppressed phenylalanine-stimulated release of CCK in a dose-related manner. The minimal concentration of trypsin needed to reduce the rise in plasma CCK was comparable to the amount required to decrease pancreatic enzyme secretion. Furthermore, intraduodenal trypsin also inhibited enzyme secretion and CCK response to oleic acid. These observations suggest that trypsin mediates feedback regulation of pancreatic enzyme secretion by inhibiting the release of CCK.

It is interesting to note that even though plasma CCK levels dropped to basal values during intraduodenal perfusion of phenylalanine and trypsin, there remained a significant increase in pancreatic enzyme secretion, which averaged 65±5.7% of that observed during perfusion of phenylalanine alone. Similar observations were made after ingestion of a test meal. Intraduodenal perfusion of trypsin suppressed postprandial increase in plasma CCK levels, although there was a small increase in pancreatic enzyme secretion. This suggests that phenylalanine- or meal-stimulated pancreatic enzyme secretion is regulated by more than one mechanism. It is conceivable that trypsin in the duodenum inhibits pancreatic enzyme secretion through suppression of CCK release, but it has no effect on other stimulatory factors, such as the enteropancreatic cholinergic reflexes that also play an important role in the intestinal phase of pancreatic enzyme secretion.

The existence of a feedback regulation of pancreatic enzyme secretion in man may have important clinical implication. It is conceivable that in patients with chronic pancreatitis, decreased pancreatic enzyme secretion may potentially result in elevated plasma CCK levels. This may reflect a failure in the feedback modulation of CCK release secondary to a deficiency of pancreatic enzyme secretion. This, in turn, may cause hyperstimulation of the pancreas and produce pain. Thus, effective enzyme replacement therapy should reduce stimulation, decrease intraductal pressure, and diminish pain. Indeed, large doses of pancreatic extract have been reported to produce relief of pain in a considerable number of patients with chronic pancreatitis (20, 30). The results of this study provide a physiological basis for this important clinical observation.

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