

Feedback Regulation of Pancreatic Enzyme Secretion as a Mechanism for Trypsin Inhibitor-Induced Hypersecretion in Rats¹ (36384)

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It has been well established that dietary trypsin inhibitors evoke an increased pancreatic enzyme secretion in rats and chicks (1-9). Pancreatic response in the intact animal was generally determined indirectly, by measuring enzyme activities in the pancreas and/or intestinal contents after feeding a diet containing trypsin inhibitors. We recently reported (10) the direct demonstration of trypsin inhibitor-stimulation of pancreatic enzyme secretion in rats in which bile-pancreatic juice was collected via a cannula. This response only occurred, however, when pancreatic juice, or trypsin (bovine), was returned to the intestine. We are reporting here additional information on the mechanism for the pancreatic response. Evidence is presented which shows that trypsin or chymotrypsin in the intestine suppresses pancreatic enzyme secretion, and that trypsin inhibitors evoke increased enzyme secretion indirectly, by counteracting the suppression produced by trypsin.

Methods and Materials. Male, Long-Evans rats, weighing between 250 and 350 g, were purchased from a local supplier (Horton's Laboratory Animals, Inc., Oakland, CA) and were fed Purina rat chow. Food, but not water, was withheld from the animals 8-10 hr before an experiment. Rats were anesthetized with urethan (100 mg/100 g of body wt, ip) and a midline incision was made to expose the duodenum. A cannula about 7

cm long (Clay-Adams, PE-50) was introduced into the bile duct where it enters the intestine, and a second cannula was introduced into the duodenum to permit direct infusion of materials to be studied. The incision with the protruding cannulas was then covered with moist cheesecloth. Body temperature was maintained with a heating pad and monitored by a thermocouple (Yellow Springs Instrument Co., Yellow Springs, OH) with a rectal temperature probe. Rectal temperature was maintained between 37 and 38°.

Combined bile-pancreatic secretions were collected in small (0.8 ml) tubes at 15 or 30 min intervals and placed in ice. Pancreatic enzyme secretion was followed by measuring chymotrypsin activity of the collected samples. The pancreas reportedly secretes the major digestive enzymes in parallel, so the secretion of any one enzyme would serve as an indicator of enzyme secretion as a whole (11). However, initially, a few samples of juice were assayed for trypsin activity, using *p*-tosyl-L-arginine ethyl ester (TAME). This additional analysis was discontinued when it became clear that trypsin activity followed the same pattern as did chymotrypsin. Chymotrypsinogen was activated by incubation of 25 μ l of bile-pancreatic juice with 475 μ l of 0.04 *M* Tris-HCl buffer (pH 8.1) containing 0.01 *M* CaCl₂ and sufficient bovine trypsin to give a final concentration of 40 μ g/ml. Activation was carried out for 15 min at 0°. Chymotrypsin activity was determined by a modification of the method of Hummel (12) using benzoyl-L-tyrosine ethyl ester (BTEE). Activity of the enzyme was expressed as milligrams of purified bovine enzyme of equal hydrolytic activity, after Geratz (13).

Intestinal infusion materials. Trypsin and

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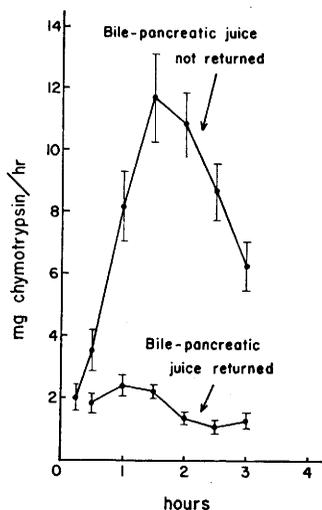


FIG. 1. Spontaneous pancreatic enzyme secretion with and without bile-pancreatic juice returned. Time scale starts with cannulation of common bile duct. Bicarbonate was replaced in animals in which bile-pancreatic juice was not returned. Chymotrypsin activity of the secreted bile-pancreatic juice served as an indication of enzyme secretion. Each point is the mean \pm SEM from 6 animals.

chymotrypsin were $2\times$ crystallized, lyophilized products obtained commercially (Worthington Biochemical Corp., Freehold, NJ). Soy hydrolysate, a tryptic digest of soy protein, was obtained from Nutritional Biochemicals Corp., Cleveland, OH. Soybean trypsin inhibitor (SBTI) was prepared in our laboratory by aqueous extraction of raw soybean flour at pH 4.1, followed by fractional precipitation of the inhibitor with ammonium sulfate. The inhibitor precipitated at 15–25% ammonium sulfate. One milligram of inhibitor preparation inhibited 1.6 mg of bovine trypsin and 0.28 mg of bovine chymotrypsin (using TAME and BTEE substrates).

Cholecystokinin-pancreozymin (CCK-PZ) was a commercial preparation (pancreozymin, Sigma Chemical Co., St. Louis, MO) containing small amounts of secretin.

Experimental procedure. Soy hydrolysate and SBTI were each dissolved or homogenized in physiological saline at a concentration of 30 mg/ml. The solutions were warmed to 37° , and infused into the intestine via the duodenal cannula at the rate of 1 ml/hr, at

15 min intervals. Trypsin and chymotrypsin (bovine) were dissolved in 0.05 *N* NaHCO_3 (pH 8.5) at 2 and 4 mg/ml, respectively, and infused at the rate of 1 ml/hr at 15 min intervals. The amount of enzyme activity infused into the intestine was within the range of basal secretion of the same enzyme in bile-pancreatic juice. The enzymes were held at 0° , and quickly brought to 37° before infusion. The effect of rat pancreatic juice was determined by returning the rat's own bile-pancreatic juice to the intestine via the duodenal cannula at 15 min intervals. In all experiments in which the bile-pancreatic juice was diverted from the animal, pancreatic juice bicarbonate was replaced by infusion of 0.05 mEq of NaHCO_3 /hr. This was estimated to be about 2 to 3 times the amount of bicarbonate normally delivered to the duodenum by the pancreatic juice.

Results. Effect of returning bile-pancreatic juice on spontaneous enzyme secretion. Figure 1 shows the enzyme secretion elicited in the absence of bile-pancreatic juice in the intestine and the suppression of this secretion when it was returned. The effect of the bile-pancreatic juice could not be attributed to acid-induced pancreatic secretion due to loss of pancreatic juice bicarbonate because bicarbonate was infused throughout the experiment. A small, but not proportional, increase in volume secretion accompanied the large increase in enzyme output.

Effect of intestinal infusion of trypsin on pancreatic enzyme secretion. In the experiments shown in Fig. 2, the effect of intraduodenal infusion of bovine trypsin on spontaneous secretion and on secretion in the presence of hydrolyzed protein (soy hydrolysate) was measured. Figure 2a shows that in the absence of bile-pancreatic secretions, trypsin alone effectively suppressed pancreatic enzyme secretion even in the presence of hydrolyzed protein, normally regarded as a powerful pancreatic enzyme secretagogue. An identical experiment was then conducted, except that the infusion of trypsin was discontinued after 2.5 hrs. The results, in Fig. 2b, clearly show that removal of trypsin greatly increased enzyme secretion. In similar experiments (14), a large increase in enzyme secre-

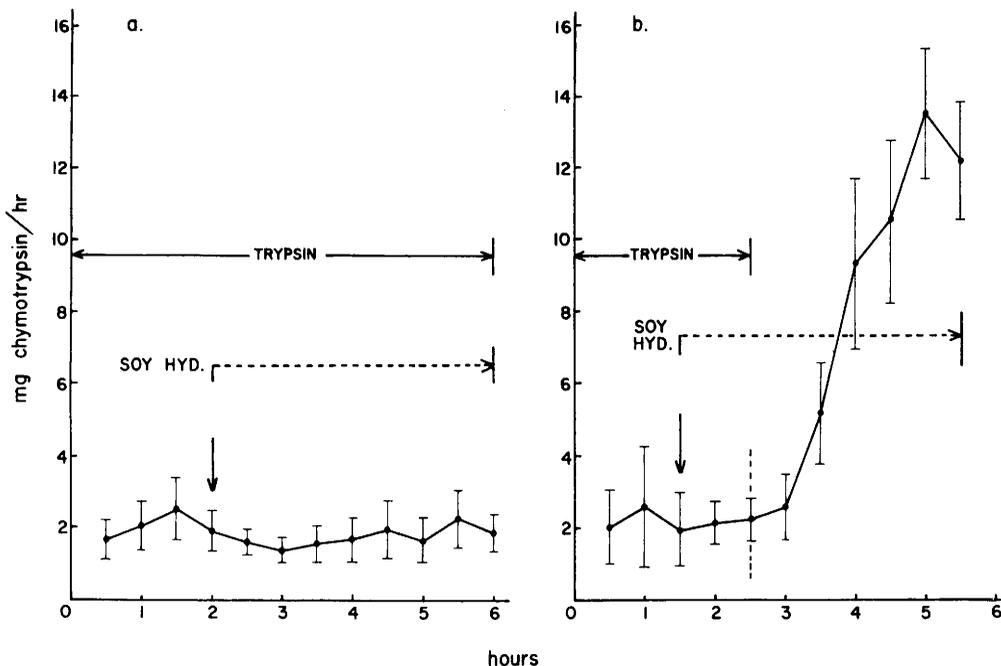


FIG. 2. Effect of trypsin on pancreatic enzyme secretion in rats with bile-pancreatic juice removed. Trypsin was infused at 2 mg/hr in 0.05 *N* NaHCO₃. Soy hydrolysate was infused at 30 mg/hr in saline. (b), Bicarbonate infusion was continued after cessation of trypsin infusion. Enzyme secretion is represented by total chymotrypsin activity of the secreted bile-pancreatic juice. Each point is the mean \pm SEM from 5 (a) or 4 (b) animals.

tion also occurred when the trypsin infusion was replaced by infusion of inactive (autoclaved) trypsin, showing that denatured trypsin did not suppress pancreatic enzyme secretion. These results indicate therefore that trypsin activity in the intestine suppresses the spontaneous increase in pancreatic enzyme secretion that occurs after removal of bile-pancreatic juice, and that hydrolyzed protein in the intestine does not stimulate increased enzyme secretion under these conditions.

Effect of trypsin inhibitors on pancreatic enzyme secretion during intestinal infusion of trypsin. This experiment was identical to the one represented in Fig. 2a, except that SBTI was infused at 4 hr. An immediate large increase in pancreatic enzyme secretion was observed (Fig. 3)³. The similarity of this

³ The increases in enzyme secretion illustrated in Figs. 1-3 were not transient, as might be suggested by the subsequent fall in enzyme output. Within 1-2 hr after maximum secretion was attained the secretory output would usually plateau or begin to rise again.

response to that observed in Fig. 2b, where trypsin infusion was discontinued, strongly indicates that the trypsin inhibitor increased secretion by combining with and neutralizing the trypsin, thereby counteracting the suppression which trypsin imposed on pancreatic secretion. In previous experiments (14) ovomucoid trypsin inhibitor produced the same results as did the SBTI. A more immediate response was evoked by SBTI than by removal of trypsin from the infusate. This was probably because some residual trypsin activity persisted after the trypsin infusion was stopped, whereas sufficient trypsin inhibitor was infused to neutralize any residual trypsin.

Effect of chymotrypsin on pancreatic enzyme secretion. To see whether chymotrypsin affected pancreatic enzyme secretion, bovine chymotrypsin was infused at 4 mg/hr throughout the experimental period. As Fig. 4 illustrates, chymotrypsin also suppressed pancreatic enzyme secretion, and this suppression, like that seen with trypsin, was reversed by infusion of 60 mg/hr of SBTI

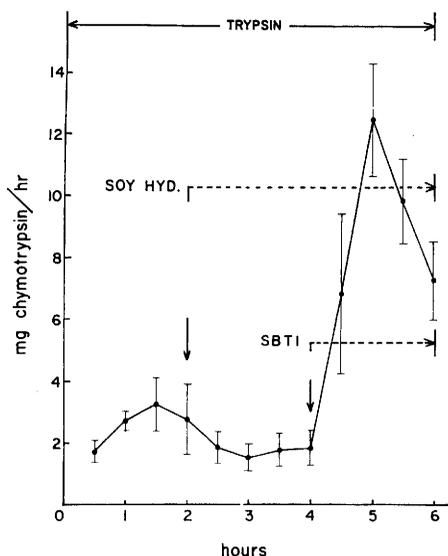


FIG. 3. Effect of trypsin inhibitor on pancreatic enzyme secretion during intestinal infusion of trypsin. Trypsin was infused at 2 mg/hr in 0.05 *N* NaHCO₃. Soybean trypsin inhibitor (SBTI) and soy hydrolysate (in saline) were each infused at 30 mg/hr. Enzyme secretion is represented by total chymotrypsin activity of the secreted bile-pancreatic juice. Each point is the mean \pm SEM from 6 rats.

(sufficient to neutralize 16 mg/hr of chymotrypsin). Thus, chymotrypsin behaves similarly to trypsin with respect to suppression of pancreatic enzyme secretion.

Pancreatic response to injected CCK-PZ during intestinal infusion of chymotrypsin and trypsin. The results shown in Fig. 5 demonstrate that the pancreatic response to CCK-PZ was not suppressed by intestinal trypsin and chymotrypsin. Intravenously injected CCK-PZ (22 Crick units) evoked a marked response from the pancreas despite the simultaneous intestinal infusion (at 8 mg/hr each) of trypsin and chymotrypsin. Spontaneous secretion, and secretion in response to hydrolyzed protein in the intestine was suppressed, however, and SBTI alleviated the suppression. These results indicate that the suppressive action of the proteolytic enzymes on pancreatic secretion was exerted at the level of the intestine, rather than at the pancreas.

Discussion. These experiments have dem-

onstrated that removal of bile-pancreatic secretions from the intestine initiates an increased output of pancreatic enzymes that is largely suppressed when either trypsin or chymotrypsin is infused. Suppression of secretion was evident even when hydrolyzed protein was in the intestine. When trypsin was in the intestine, simultaneous infusion of trypsin inhibitors evoked increased enzyme secretion; a similar response was obtained by discontinuing the infusion of trypsin (Figs. 3 and 2b). It appeared, therefore, that the increased enzyme secretion induced by trypsin inhibitor was due to its effective removal of trypsin activity from the intestine by combining with trypsin, rather than to any specific secretagogue effect of the trypsin inhibitor itself.

These results provide an explanation for the effects of dietary trypsin inhibitors on rats and chicks. In both species, trypsin inhibitors stimulate increased pancreatic enzyme secretion, determined indirectly (1-9). In addition, chicks fed trypsin inhibitors show

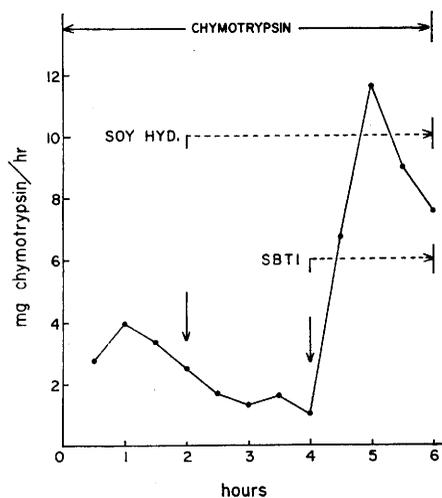


FIG. 4. Effect of trypsin inhibitor on pancreatic enzyme secretion during intestinal infusion of chymotrypsin. Chymotrypsin was infused at 4 mg/hr in 0.05 *N* NaHCO₃. Soybean trypsin inhibitor (SBTI) and soy hydrolysate were infused at 60 and 30 mg/hr, respectively. SBTI inhibited 0.28 mg of chymotrypsin/mg of inhibitor. Enzyme secretion is represented by total chymotrypsin activity of the secreted bile-pancreatic juice. Each point is the average of 2 animals.

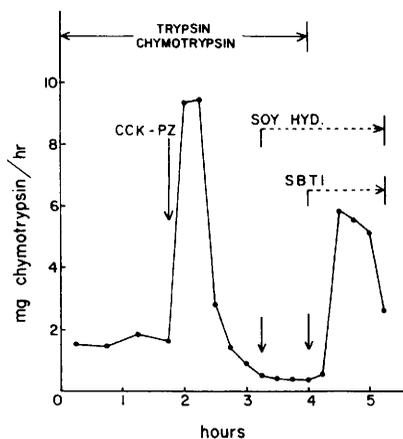


FIG. 5. Pancreatic response to injected CCK-PZ during intestinal infusion of chymotrypsin and trypsin. Trypsin and chymotrypsin were infused together at 8 mg/hr each in 0.05 *N* NaHCO₃. Soybean trypsin inhibitor and soy hydrolysate were infused at 60 and 30 mg/hr, respectively. Enzyme secretion is represented by total chymotrypsin activity of the secreted bile-pancreatic juice. Only one animal was used in this experiment.

a marked depression in intestinal proteolytic activity for several hours after ingestion (8, 15, 16) and recently a similar effect has been demonstrated in rats (5, 14). The reduced intestinal proteolytic activity in chicks fed trypsin inhibitors led some investigators (8, 17) to suggest that the increased pancreatic secretion was a compensatory response by the pancreas to restore intestinal proteolytic activity, which had been lowered by the trypsin inhibitors in the intestine.

This hypothesis was difficult to apply to the rat because in this species it appeared that intestinal proteolytic activity was increased greatly within a short time after feeding of the trypsin inhibitor (1, 3, 4). However, it should be noted that proteolytic activity was determined by hemoglobin digestion, which measures total proteolytic activity of all the digestive enzymes (trypsin, chymotrypsin, carboxypeptidases). Therefore, depression of one proteolytic enzyme could be masked by elevation of another. This was shown to be the case by Green and Lyman (5) who used specific enzyme substrates and were able to show that intestinal trypsin activity in SBTI-fed rats was greatly lowered,

and remained below control values for up to 8 hr after feeding. Intestinal chymotrypsin activity, however, appeared to be greatly increased. Evidence obtained by Green (14) indicates that this apparent increase in chymotrypsin activity (which probably accounted for the increased proteolytic activity reported by others) was an artifact produced by the method used in obtaining and/or preparing the intestinal contents for analysis. It was found that intestinal chymotrypsin in SBTI-fed rats was largely in its inactive precursor form, chymotrypsinogen, and was apparently converted to chymotrypsin during the preparation of the intestinal contents for analysis. Intestinal contents of SBTI-fed rats collected under conditions which minimized activation of chymotrypsinogen did not show increased chymotrypsin activity, until the samples were subsequently activated under suitable conditions. These results indicated that, in the intact animal, trypsin inhibitors may impair chymotrypsinogen activation (which is trypsin dependent) by blocking intestinal trypsin activity.

If, as suggested above, dietary trypsin inhibitors reduce both trypsin and chymotrypsin activities in the intestine, then the pancreatic response to trypsin inhibitors may be explained. By reducing intestinal proteolytic activity, trypsin inhibitors would be removing the suppression by trypsin and chymotrypsin of pancreatic enzyme secretion and increased enzyme output would follow. Thus, in the rat, pancreatic enzyme secretion appears to be subject to negative feedback control by intestinal trypsin and chymotrypsin activities. This probably applies to the chick as well.

If chicks are fed trypsin inhibitors for a prolonged period of time, the pancreas (through hypertrophy and increased zymogen synthesis) can apparently secrete enough additional enzyme to neutralize the inhibitor and restore normal intestinal proteolytic activity (8, 15, 16). This observation led Lepkovsky *et al.* (8) to suggest that the secretion of pancreatic juice in the chick might be under some sort of homeostatic feedback control which tended to restore the level of intestinal proteolytic activity to nor-

mal when it was disturbed. No direct evidence for such a control mechanism was presented, however. Our results provide direct evidence for a control mechanism by which homeostasis of intestinal proteolytic activity could be maintained. Such a mechanism also would explain the spontaneous hypersecretion observed by Grossman (18) in chronic fistula rats. The fact that intraduodenal infusion of amino acids could not augment the secretion suggests that the pancreas may have been secreting maximally, due to the absence of feedback inhibition from intestinal proteolytic activity.

The mechanism(s) by which trypsin or chymotrypsin suppresses pancreatic enzyme secretion is not known. The experiment illustrated by Fig. 5 indicated that intestinal proteolytic activity suppressed pancreatic enzyme secretion by interfering with the pancreatic response to *intestinal* stimuli, rather than by direct action on the pancreas. Pancreatic enzyme secretion resulting from intestinal stimuli is presumably mediated largely by the hormone CCK-PZ located in the intestinal mucosa (19). Quite possibly trypsin and chymotrypsin may interfere with the release of CCK-PZ from the intestine. This would be consistent with studies indicating that trypsin inhibitor-induced pancreatic secretion is mediated by CCK-PZ (3, 20-22). We suggest, therefore, that intestinal proteolytic activity is an important factor controlling the release of CCK-PZ in the rat.

It is not known whether feedback inhibition from intestinal proteolytic enzymes exists in higher animals. Results obtained by Goldberg, Campbell, and Roy (23) suggest that intestinal trypsin and chymotrypsin may suppress pancreatic enzyme secretion in man. In the dog, however, Cooke, Nahrwold, and Grossman (24) found that the pancreatic enzyme response to feeding was unchanged whether the pancreatic juice was drained off or returned. Clearly, the problem should be reinvestigated in higher animals.

Nonproteolytic pancreatic enzymes are probably not involved in inhibition of pancreatic secretion. For example, if lipase or amylase appreciably suppressed pancreatic enzyme secretion, then one would not expect

inhibition of proteolytic enzymes alone (*i.e.*, by feeding trypsin inhibitors) to result in a large pancreatic response in intact animals.

Summary. The mechanism by which dietary trypsin inhibitor induces excessive pancreatic secretion was investigated in rats with bile-pancreatic duct fistulas. It was found that removal of bile-pancreatic juice from the intestine resulted in a large increase in pancreatic enzyme secretion. Infusion of trypsin or chymotrypsin as well as bile-pancreatic juice suppressed the secretion of pancreatic enzymes. When trypsin was present in the intestine, a large pancreatic enzyme response was obtained by infusion of trypsin inhibitors. A similar increase in pancreatic enzyme output was evoked when trypsin infusion was stopped. The results indicated that pancreatic enzyme secretion in the rat is subject to feedback inhibition from intestinal trypsin and chymotrypsin, and that trypsin inhibitors stimulate pancreatic enzyme secretion indirectly, by binding or neutralizing trypsin and thereby removing its feedback inhibition.

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