Chymotrypsin Inhibitor Stimulation of Pancreatic Enzyme Secretion in the Rat[1] (35332)

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An increase in pancreatic enzyme secretion and pancreatic hypertrophy following the ingestion of various trypsin inhibitors are well established phenomena in rats and chicks (1, 2). The diverse origins of the many trypsin inhibitors that elicit the pancreatic response—their dissimilarity in molecular weight, heat lability, and other physicochemical properities—suggest that their physiological effect, when fed to animals, must be related to their common property, the ability to inhibit tryptic hydrolysis.

No studies, to our knowledge, have been published which have described the pancreatic response to inhibitors of the other major pancreatic proteolytic enzyme, chymotrypsin. The reason for this lies probably in the fact that, until recently, no "pure" chymotrypsin inhibitor (*i.e.*, one which is devoid of trypsin inhibitor or other enzyme inhibitor activity) suitable for dietary studies was available. With the discovery and isolation by Ryan and Balls (3) of a chymotrypsin inhibitor from potatoes, such a study became feasible. The investigation described below compared the acute exocrine pancreatic response of rats fed a single dose of potato chymotrypsin inhibitor (PCI) to that of rats fed either soy bean trypsin inhibitor (SBTI) or the control diet containing casein.

Materials and Methods. Male, Long-Evans rats weighing 90–110 g, were purchased from a local supplier (Simonsen, Gilroy, California) and were maintained *ad libitum* on Purina Laboratory Rat Chow. Food was removed from the animals 24 hr before an experiment. In each experiment, the inhibitor or protein to be fed was mixed with 2 g of a basic, 18% casein semipurified diet containing 71% sucrose and no celluflour (4) and with enough water to form a viscous slurry, which was then placed in the animal by stomach tube. The rats were divided into 4 groups: Group I received the basic diet plus 25 mg/100 g of body weight of PCI; group II was fed the diet plus 42 mg/100 g of body weight of SBTI; group III, the control group, was fed the diet plus 50 mg/100 g of body weight of purified casein; group IV was not fed following the 24-hr fast, and was used to establish the fasting levels of the enzymes assayed. The quantities of the inhibitors fed in each case were identical in terms of the total amount of enzyme they inhibited (*i.e.*, 25 mg of PCI and 42 mg of SBTI inhibited 42 mg of bovine α-chymotrypsin and 42 mg of bovine trypsin, respectively). The amount of SBTI fed was known from previous experience to be more than adequate to elicit a maximal response from the rat pancreas.

Groups I, II, and III were sacrificed by decapitation at 2, 4, 6, and 8 hr after receiving the diets by stomach-tube, and group IV was killed following the 24-hr fast. The pancreas and intestinal contents were immediately removed. The pancreas was dissected free of visible fat and lymph nodes under a dissecting microscope, frozen, and lyophilized. Intestinal contents were collected by dividing the intestine into 4 sections and washing each section with 5 ml of ice-cold water, and quickly freezing the washes before lyophilization. The lyophilized material was prepared for enzyme assays by homogenizing appropriate aliquots with a Duall Teflon tissue grinder. Pancreases were homogenized in 0.9% cold NaCl; intestinal contents were homogenized in cold 0.1 *M* imidazole buffer, pH 7.4, containing 0.1 *M* NaCl and 0.1% Triton X-100. This buffer system has been

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shown (5) to greatly enhance the recovery of lipase from intestinal debris, in addition to preventing bacterial growth.

Enzyme assays. Trypsin and chymotrypsin were assayed by a modification of the methods described by Hummel (6) using *p*-tosyl-L-arginine methyl ester (TAME) and benzoyl-L-tyrosine ethyl ester (BTEE) as substrates for trypsin and chymotrypsin, respectively. Pancreatic zymogens were activated by incubation with purified enterokinase (Nutritional Biochemicals Corp., Cleveland, Ohio) at 0° for 24 hr. The incubation mixture contained 1 mg/ml of lyophilized pancreas and 2 mg/ml of purified enterokinase in 0.04 *M* Tris buffer, pH 8.1, containing 0.01 *M* $CaCl_2$.

Lipase activity was determined by titrating the fatty acids liberated by the hydrolysis of an emulsion of commercial olive oil (7), and is expressed as milliequivalents of NaOH required to back-titrate the incubation mixture to pH 9.3.

Source of inhibitors. The chymotrypsin inhibitor was prepared and was generously supplied to us by Dr. C. A. Ryan of Washington State University. Assays were performed to determine its inhibitory potency toward chymotrypsin and trypsin. Analysis with either casein (Fig. 1) or synthetic substrates (TAME and BTEE) revealed the chymotrypsin inhibitor preparation to be almost completely devoid of trypsin inhibitory activity, while inhibiting 1.7 times its weight of bovine α-chymotrypsin. The material was also reported to be free of any carboxypeptidase inhibitor activity (personal communication from Dr. Ryan).

The SBTI (trypsin inhibitor of Kunitz) was a 2× crystallized product (Worthington Biochemical Corp., Freehold, New Jersey), which inhibited equal weights of bovine trypsin (1:1 ratio) and demonstrated no significant chymotrypsin inhibitor activity.

Averaged results were subjected to statistical evaluation using the Student's *t* test, and differences in mean values yielding $p<0.05$ were considered significant. Each point in the figures represents the average of individual analyses from 3 rats. The only exceptions are the 2- and 6-hr points from PCI-fed animals which are averages from 2 animals.

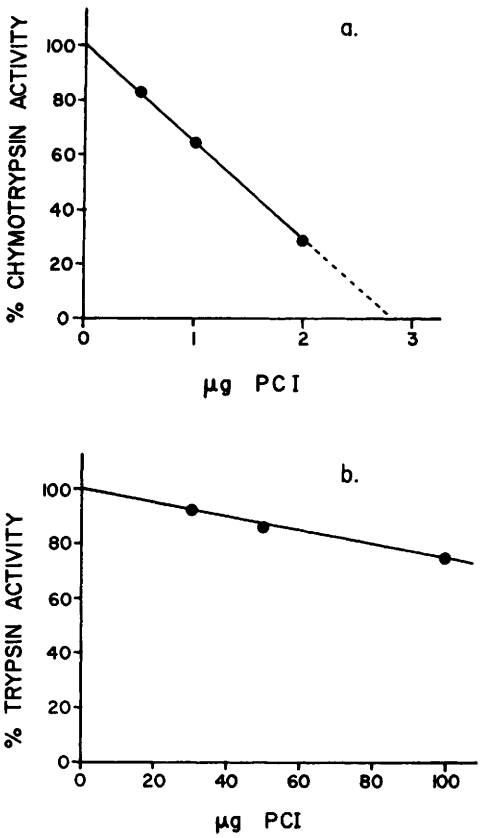

FIG. 1. Inhibition of trypsin and chymotrypsin activity by potato chymotrypsin inhibitor (PCI): (a) chymotrypsin inhibition by PCI against 5 μg of chymotrypsin with casein as substrate; 1.5 μg of inhibitor required for 50% inhibition of chymotrypsin. (b) trypsin inhibition by PCI against 10 μg of trypsin with casein as substrate; 190 μg of inhibitor required for 50% inhibition of trypsin.

Results and Discussion. Pancreatic tissue activity. Pancreatic response to the feeding of the inhibitors was assessed by the degree of depletion, over a period of time, of trypsin, chymotrypsin, and lipase activities in the pancreatic tissue compared to controls fed the casein. Amylase was not included, since it has been shown previously that protease, amylase, and lipase activities are secreted in parallel in response to trypsin inhibitors (1). Figure 2 illustrates the response of these enzymes at 2, 4, 6, and 8 hr after feeding either SBTI, PCI, or the control diet. The response obtained with SBTI was typical for the rat (1, 8), and reflected the strong pan-

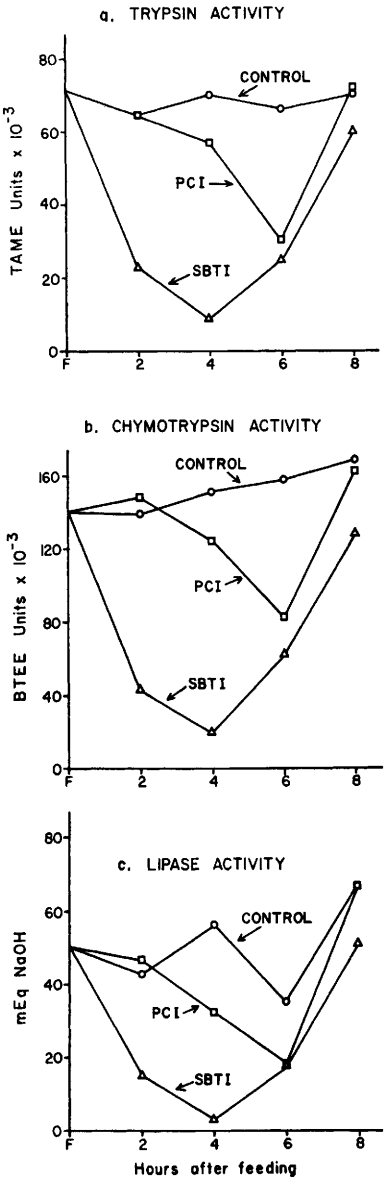

FIG. 2. Pancreatic enzyme activity of rats after a single feeding of either potato chymotrypsin inhibitor (PCI), soybean trypsin inhibitor (SBTI) or control diet containing casein: The abscissa represents hours postfeeding. Values at F are for fasted animals that received no subsequent feeding. Trypsin (2a) and chymotrypsin (2b) activities are expressed as TAME and BTEE units $\times 10^{-3}$; lipase activity (2c) is expressed as mEq/hr. All enzymes are expressed as activity/pancreas/100 g of body weight.

creatic stimulatory properties of this dietary trypsin inhibitor compared with casein. By 2 hr after feeding (Fig. 2a, b), pancreatic trypsin and chymotrypsin activities were less than 40% of control values ($p<0.05$). Maximum depletion occurred between 2 and 6 hr, at which time the chymotrypsin and trypsin activities of the SBTI group were only 14% of control values ($p<0.05$). By 8 hr, the pancreases had been repleted, presumably because by this time the trypsin inhibitor had passed through the intestinal tract, and the differences between the two groups were no longer significant. Lipase activity (Fig. 2c) followed essentially the same pattern as trypsin and chymotrypsin for the two groups, except for some irregularity in the 6-hr control values.

In contrast, the pancreatic response to the feeding of PCI was not nearly as pronounced as with SBTI, and the time of maximal depletion was delayed (Fig. 2a, b, c). By 2 hr after feeding, there were no significant differences between the controls and the PCI-fed group for any enzyme measured, but by 6 hr, there was a significant depletion of trypsin and chymotrypsin activities [44 and 52% of control values, respectively, ($p<0.01$) and of lipase (52% of control values ($p<0.05$))]. By 8 hr, the enzyme activities for animals fed PCI had also returned to control values.

Enzyme activity of intestinal contents. Lipase was assayed in these experiments to circumvent a problem which occurs when pancreatic proteolytic enzyme inhibitors are fed; namely, the inhibitors can combine with, and may inhibit the activity of the enzymes secreted into the intestine, thereby obscuring the actual amounts of enzyme present. Also, inhibition of trypsin can indirectly influence the activity of other enzymes, *e.g.*, chymotrypsin and carboxypeptidase, since their zymogens are activated by trypsin. A few samples of intestinal contents were analyzed for amylase activity which also displayed a pattern quite similar to that of lipase.

The greatly increased lipase activity in the intestinal contents of rats fed SBTI reflected the depletion of the same enzyme from the pancreas (Fig. 3). By 2 hr after

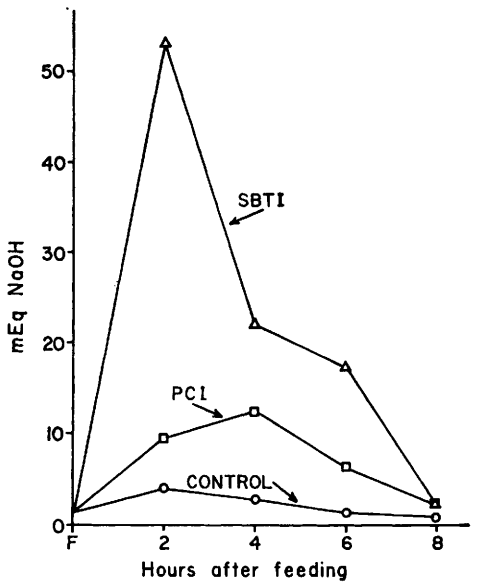

FIG. 3. Lipase activity of intestinal contents of rats after a single feeding of either potato chymotrypsin inhibitor (PCI), soybean trypsin inhibitor (SBTI) or control diet containing casein: The abscissa represents hours postfeeding. Values at F are for fasted animals that received no subsequent feeding. Lipase is expressed as activity/total intestinal contents/100 g of body weight.

feeding SBTI, lipase activty had increased 10-fold over that of controls ($p<0.001$). The activity then progressively decreased until by 8 hr it had returned to control values. Animals fed PCI exhibited a much less, but still significant, increase in intestinal lipase activity as compared to controls. By 4 hours, there was nearly a 5-fold increase ($p<0.01$) over controls, which by 8 hr had also returned to control levels.

The assay of intestinal trypsin and chymotrypsin activity revealed a pattern of enzyme activity different from that of lipase (Fig. 4). Chymotrypsin activity (Fig. 4a) in SBTI-fed animals rose to levels almost 10 times that of controls by 4 hr, and was nearly the inverse of the pancreatic depletion of chymotrypsin in the same group. When rats were fed PCI, however, intestinal chymotrypsin activity was lower than that of controls, and remained so throughout the experiment. Differences between controls and animals fed PCI were significant at 4 and 6 hr ($p<0.05$). Apparently, the PCI in the intestinal tract had neutralized virtually all of the chymotrypsin present, obscuring any increases or other changes in chymotrypsin activity which may have occurred. This almost total depression of chymotrypsin activity in the gut provides good evidence that substantial amounts of PCI survived gastric digestion.

The patterns of intestinal trypsin activity, when either of the two inhibitors were fed, are shown in Fig. 4b. Rats fed PCI showed an increase in trypsin activity that was only slightly greater than control values at the

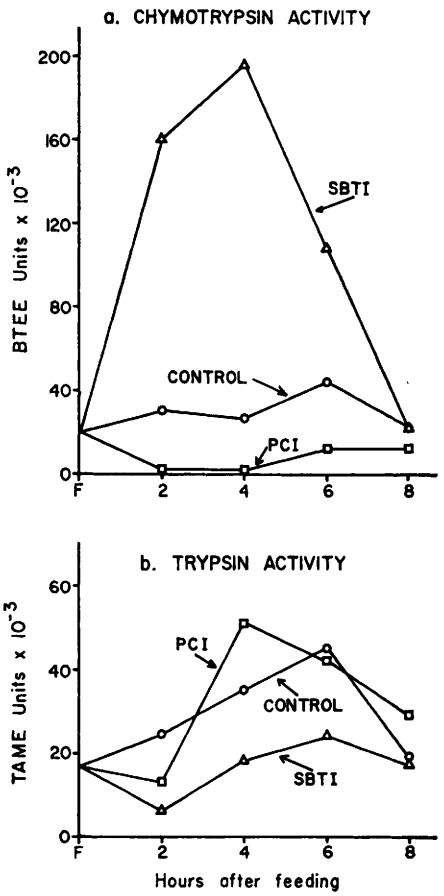

FIG. 4. Chymotrypsin and trypsin activity of intestinal contents of rats after a single feeding of either potato chymotrypsin inhibitor (PCI), soybean trypsin inhibitor (SBTI), or control diet containing casein: Enzyme activity is expressed as TAME or BTEE units $\times 10^{-3}$/total intestinal contents/100 g of body weight. The abscissa represents hours postfeeding. Values at F are for fasted animals that received no subsequent feeding.

4-hr period, but due to wide variation in the control enzyme activities the difference was not statistically significant. The SBTI-fed animals, on the other hand, had depressed intestinal trypsin activity significantly ($p<$ 0.05) below that of controls at 2, 4, and 6 hr. As was the case with intestinal chymotrypsin activity of PCI-fed rats, the SBTI in the intestinal tract completely neutralized any excess trypsin secreted by the hyperstimulated pancreas.

It appears from these data that PCI does evoke an increased pancreatic secretion of enzymes. The response, however, is not nearly so pronounced as with rats fed SBTI. Furthermore, the time of maximal stimulation by PCI occurs later, that is, between 4 and 8 hr after feeding the inhibitor; whereas when SBTI is fed, maximum stimulation is obtained between 2 and 6 hr. The data also indicate that the large increases in intestinal proteolytic activity previously observed when total proteolytic activity was measured by hemoglobin digestion (1, 8) in rats after SBTI was fed were due mainly to increased chymotrypsin activity, rather than to trypsin.

The mechanism by which proteolytic enzyme inhibitors stimulate pancreatic enzyme secretion is not known. It has been suggested that the pancreas may respond to the inhibition of intestinal proteolytic activity by secreting an excessive amount of enzyme (9). If this is so, it would appear that, in light of the results presented above (Fig. 4), the inhibition of either chymotrypsin or trypsin was sufficient to elicit and maintain a pancreatic response. However, Lyman *et al.* (1) have demonstrated that the feeding of a large excess of trypsin along with SBTI did not diminish the pancreatic response compared to rats fed SBTI alone. Under these conditions, one would not expect that a deficiency of intestinal trypsin would occur, so the question of what triggers the secretion still remains unclear. Geratz (10) has suggested that trypsin inhibitors may exert their effect on the pancreas independently of any inhibition of trypsin activity, but may act instead by attaching to unrelated receptors in the intestinal mucosa. These receptors, he suggests, would share structural similarities with the trypsin molecule and could be important in the release of pancreozymin. If this hypothesis is valid, it would have to be broadened to explain the results obtained with the feeding of PCI. The hypothetical "trypsin-like" receptors in the mucosa would have to be envisioned as interacting with chymotrypsin inhibitors also, or perhaps separate "chymotrypsin-like" receptors exist. In any case, it remains open to speculation why an inhibitor which interacts with trypsin, or with "trypsin-like" receptors, should evoke a greater pancreatic response than one which interacts with chymotrypsin, or with "chymotrypsin-like" receptors. Therefore, we are still unable to say with any certainty how either type of inhibitor is able to stimulate pancreatic secretion above that produced by normal dietary secretagogues.

Summary. Experiments were conducted to investigate the effect of dietary chymotrypsin inhibitor on pancreatic enzyme secretion in the rat, and to compare the response to that evoked by soy bean trypsin inhibitor.

Potato chymotrypsin inhibitor evoked a moderately exaggerated pancreatic response compared to casein-fed controls, as demonstrated by a depletion of pancreatic trypsin, chymotrypsin, and lipase activities, and a concomitant increase in the activities of intestinal trypsin and lipase. The PCI-induced response was not nearly as pronounced as that produced by SBTI, in addition to being somewhat delayed.

Intestinal trypsin activity in SBTI-fed rats, and chymotrypsin activity in PCI-fed rats, were much less than in controls—often lower than in the fasting state—reflecting an almost total inhibition of the enzymes by their inhibitors as they passed through the gut. In rats fed SBTI, the large increase in intestinal proteolytic enzyme activity was due almost exclusively to chymotrypsin.

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