Protein, as a Regulator of Pancreatic Enzyme Secretion in the Rat[1] (37199)

GARY M. GREEN, BARBARA ANN OLDS, GLENDA MATTHEWS, AND R. L. LYMAN
Department of Nutritional Sciences, University of California, Berkeley, California 94720

We recently reported (1) that pancreatic enzyme secretion in anesthetized rats was suppressed by trypsin, chymotrypsin, or bile-pancreatic juice in the intestine. Removal of proteolytic activity from the intestine by diversion of bile-pancreatic juice or by intestinal infusion of soybean trypsin inhibitor produced a large increase in pancreatic enzyme output. It was suggested that this "negative feedback control" of pancreatic enzyme secretion could be the mechanism by which dietary trypsin inhibitors induced excessive pancreatic enzyme secretion in rats (2, 3).

The purpose of the present study was to see whether pancreatic response to normal foodstuffs could be explained by a similar mechanism of negative feedback control. Since protein is the natural substrate for trypsin and chymotrpsin, we conducted a preliminary experiment in anesthetized rats (Fig. 1) which showed that protein stimulated a marked increase in pancreatic enzyme secretion when trypsin was present in the intestine, whereas hydrolyzed protein (soy hydrolysate) was much less effective under the same conditions. In the present study we have extended this observation by comparing pancreatic enzyme secretion induced by intact protein, protein hydrolysates and soybean trypsin inhibitor, when bile-pancreatic juice was being returned to the intestine of the conscious rat.

Methodology. Male, Wistar rats (Hilltop Lab Animals, Chatsworth, CA), weighing 300–350 g, were fed Purina rat chow. Food, but not water, was withheld from the animals 8 hr before the operation. They were then anesthetized with methoxyflurane (Metafane, Pitman-Moore Inc., Washington Crossing, NJ) which was delivered to them through a plastic face mask by means of a vaporizer connected to an oxygen cylinder. A midline abdominal incision was made and the duodenum was exposed. The bile duct was cannulated at the point of entry into the intestine. The cannula consisted of a 12 in. length of Silastic Medical Grade tubing (Dow-Corning), 0.025 in. i.d. × 0.047 in. o.d., to which a 5 mm piece of Teflon (Becton-Dickinson & Co.) tubing (0.027 × 0.039 in.) was attached. The beveled tip of the Teflon piece was inserted toward the liver only a few millimeters into the bile duct and secured with several ligatures. Duodenal cannulas were established by inserting a piece of Silastic tubing through an incision in the intestine, and securing it by purse-string ligatures. In some cases, an additional duodenal cannula was provided to allow simultaneous infusion of foodstuffs and bile-pancreatic juice. A gastric cannula was provided by insertion of a larger (0.030 × 0.065 in.) piece of Silastic tubing into the squamous portion of the stomach, and securing it by purse-string ligatures. Before closing the abdominal incision about 60 mg of penicillin powder (Pen-Na, Penicillin-G, 1650 units/mg, Sigma Chemical Co., St. Louis, MO) were placed into the abdominal cavity. All cannulas were brought out through the incision and passed under the skin to eventually exit dorsally near the tail. Aseptic precautions were practiced throughout the operation. The animals were placed in modified Bollman restraint cages and allowed to recover. Routinely, a 48-hr recovery period elapsed before experiments were performed. Rat chow was provided during this time and the animals usually began eating

[1] Supported in part by Research Fellowship F02AM5086801, National Institutes of Health, Bethesda, MD, awarded to G. M. G.

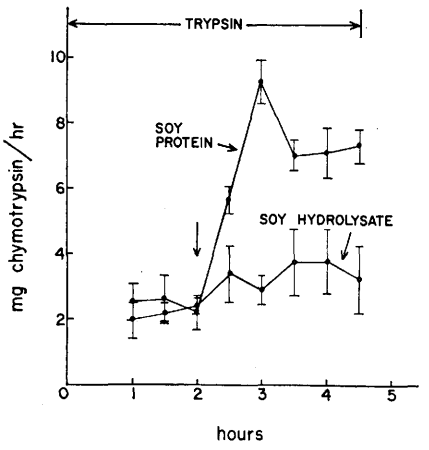

FIG. 1. Effect of infusing trypsin on pancreatic enzyme secretion in response to protein or hydrolyzed protein in the intestine. Bovine trypsin was infused into the duodenum in place of bile-pancreatic juice at 4 mg/hr (1 ml/hr) in 0.05 *N* $NaHCO_3$. Soy protein and soy hydrolysate were infused as 6% solutions in saline at 1 ml/hr for 3 hr. Pancreatic enzyme secretion is expressed as the total chymotrypsin activity of the secreted bile-pancreatic juice. The experiment was conducted while the rat was anesthetized with methoxyflurane. (Metafane). Each point is the mean ± SEM from 5 animals.

within a few hours after regaining consciousness. The bile-pancreatic juice, which began to flow immediately after cannulation of the common bile duct, was returned to the intestine during recovery and in between experiments by joining the bile-duct cannula and duodenal cannula. Basal flow (when secretions were returned) averaged about 2 ml/hr shortly after recovery and did not increase significantly during the week following the operation. The combined bile and pancreatic secretions were collected to avoid problems associated with the very low flow rate when pure pancreatic juice is collected in the rat. Any effect on pancreatic secretion was due to the proteolytic enzymes in pancreatic juice, not to the bile.

Pancreatic enzyme secretion was followed by measuring chymotrypsin activity of the secreted bile-pancreatic juice. In some experiments, trypsin, amylase and total protein in the secretions were also assayed, all of which were found to be secreted in parallel with chymotrypsin. Chymotrypsinogen was activated by incubation of 25 μl of bile-pancreatic juice with 475 μl of 0.04 *M* Tris–HCl buffer (pH 8.1) containing 0.01 *M* $CaCl_2$ and sufficient bovine trypsin to give a final concentration of 40 μg/ml. Activation was carried out for 15 min at 0°. Chymotrypsin activity was determined by a modification of the method of Hummel (4) using benzoyl-L-tyrosine ethyl ester (BTEE). Chymotrypsin activity of the secretions was expressed as milligrams of purified bovine enzyme of equal esterolytic activity.

Materials. Casein was obtained commercially as Casein Hammersten (Nutritional Biochemicals Corp., Cleveland, OH). Casein hydrolysates were obtained from Sheffield Chemical Co., Union, NJ. The acid hydrolyzed casein (Sheffield Hy-CASE SF, salt-free) contained 75% of its total nitrogen as α-amino nitrogen (75% αN/TN). Enzymatic hydrolyzates of casein contained 54% (Sheffield N-Z Amine AW) and 35% (Sheffield Pepticase) of their total nitrogen as α-amino nitrogen. Soybean trypsin inhibitor was prepared in our laboratory as described previously (1). One milligram of inhibitor preparation inhibited 1.7 mg of bovine trypsin and 0.47 mg of bovine chymotrypsin. Trypsin and chymotrypsin used as standards for enzyme activity determinations or for intestinal infusion were obtained from Worthington Biochemical Corp. (Freehold, NJ) as the salt-free crystalline enzymes.

Experimental Procedure. Casein was prepared for infusion by homogenizing appropriate amounts in water using a Duall tissue grinder with a glass rod. Casein hydrolysates were simply dissolved in water. Substances to be infused were brought to 5 ml and infused as one 5-ml portion when introduced into the stomach, or as five 1-ml portions, 5 min apart, when introduced into the intestine. Bile-pancreatic secretions were continuously returned to the intestine by collecting 15-min samples and returning them to the duodenum by syringe pump during the following 15-min collection period. A 25-μl aliquot of bile-pancreatic juice was taken every 15 min for assay. Rats were routinely fasted for about 8 hr before experiments were begun. In experiments where two different stimulants were tested in the same animal on the same day, the weaker stimulant was given

first, followed by the stronger stimulant several hours later. Experiments were conducted from the third through the seventh postoperative day, after which the animal was sacrificed.

Results. 1. Effect of casein and casein hydrolysate on pancreatic enzyme secretion during return of bile-pancreatic juice. In the experiment illustrated in Fig. 2, 250 mg of casein or casein hydrolysate in 5 ml of water was introduced into the stomach via the gastric cannula. Intact casein evoked a considerably larger increase in enzyme secretion than did either acid or enzymatic hydrolysates of casein. For comparison, the effect of administering 5 ml of water is also shown. It should be noted that basal enzyme secretion in these

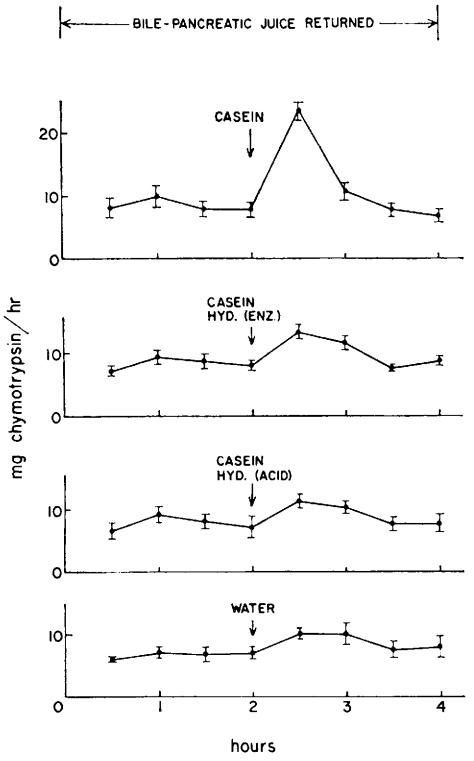

FIG. 2. Effect of casein or casein hydrolysates on pancreatic enzyme secretion while bile-pancreatic juice was being returned. Two-hundred and fifty milligrams (in 5 ml H_2O) of casein or casein hydrolysate were introduced into the *stomach* via the gastric cannula. Pancreatic enzyme secretion is expressed as total chymotrypsin activity of the secreted bile-pancreatic juice. Each point is the mean ± SEM from 6 animals.

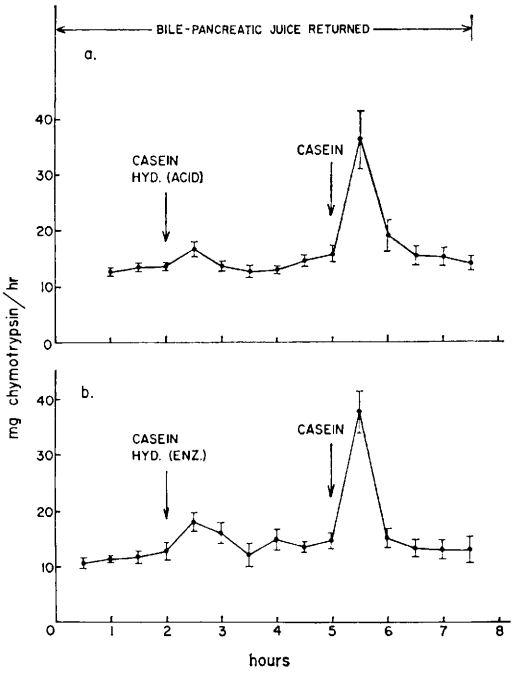

FIG. 3. Effect of casein or casein hydrolysate on pancreatic enzyme secretion during return of bile-pancreatic juice. Two-hundred and fifty milligrams (5 ml) of casein or casein hydrolysate were introduced into the *intestine* via the duodenal cannula. Pancreatic enzyme secretion is expressed as total chymotrypsin activity of the secreted bile-pancreatic juice. Each point is the mean ± SEM from 6 animals.

animals was 3- to 4-fold that seen in the anesthetized animals (Fig. 1). Figure 3 shows the results of introducing 250 mg (5 ml) of casein hydrolysate or casein into the intestine, during return of bile-pancreatic juice. Again, intact casein evoked a marked increase in enzyme secretion compared to either the acid or enzymatically hydrolyzed casein preparations. The pancreatic hydrolysate of casein (N-Z Amine AW, 54% αN/TN) appeared to have evoked a slightly larger increase than the acid hydrolysate (Hy-Case SF, 75% αN/TN) when the foodstuffs were infused directly into the intestine.

2. Effect of soybean trypsin inhibitor (SBTI) on pancreatic enzyme secretion during return of bile-pancreatic juice. The animals in this experiment received 150 mg of SBTI (ca. 50 mg/100 g of body wt) via a gastric cannula while bile-pancreatic juice was being

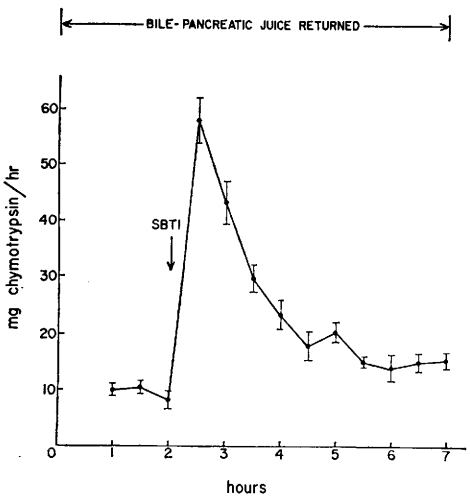

FIG. 4. Effect of soybean trypsin inhibitor on pancreatic enzyme secretion during return of bile-pancreatic juice. Soybean trypsin inhibitor (150 mg in 5 ml H_2O) was introduced into the stomach via a gastric cannula. Pancreatic enzyme secretion is expressed as total chymotrypsin activity of the secreted juice. Each point is the mean ± SEM from 5 rats.

returned. Soybean trypsin inhibitor (Fig. 4) elicited a much greater increase in enzyme secretion than that obtained with the larger amount of casein (250 mg). This result would be expected in view of the much larger pancreatic response evoked by SBTI than that elicited by protein in intact rats when measured by indirect procedures (2, 3).

3. Effect of diverting bile-pancreatic juice from the intestine on pancreatic enzyme secretion. In a previous study (1), we showed that diversion of bile-pancreatic juice in anesthetized rats led to an immediate, large, prolonged increase in pancreatic enzyme secretion. This spontaneous increase was prevented by returning bovine trypsin and/or chymotrypsin to the intestine. The results illustrated in Fig. 5 show that diversion of bile-pancreatic juice in the conscious rat also leads to an immediate large increase in the rate of pancreatic enzyme secretion, which remains elevated until the secretions are again returned to the intestine. In experiments not shown, replacement of bile-pancreatic juice in these rats by solutions of bovine trypsin and chymotrypsin having an activity approximately equal to that in the bile-pancreatic juice suppressed the spontaneous enzyme increase seen in Fig. 5. Introduction of foodstuffs (protein, protein hydrolysates and SB TI) during diversion of bile-pancreatic juice did not produce any significant increases in enzyme secretion.

Discussion. These experiments on conscious rats have shown that the pancreatic enzyme secretory response to certain substances is modified by the presence or absence of bile-pancreatic juice in the intestine. When bile-pancreatic juice is removed from the intestine, hypersecretion of the pancreatic enzymes occurs; under these conditions, introduction of protein, protein hydrolysates or SBTI does not augment enzyme secretion. When bile-pancreatic juice is returned to the intestine, enzyme secretion is suppressed. Introduction of protein or hydrolysates during these more physiological conditions shows that intact protein is a much more effective stimulant for pancreatic enzyme secretion in the rat than is hydrolyzed protein. Earlier experiments on dogs have led to the belief that products of protein digestion, such as peptones or amino acids, in the intestine are the most important intraluminal stimulants for pancreatic enzyme secretion (5), while intact protein is not regarded as a potent secretagogue (6). However, in the dog experiments, pancreatic juice was excluded from the intestine during introduction of the foodstuff, which may have influenced the pancreatic response.

Why should intact protein be a more potent secretagogue than hydrolyzed protein when pancreatic juice, or trypsin (Fig. 1), is present in the intestine? We suggest that intact protein stimulates pancreatic enzyme secretion by a mechanism similar to that proposed by us for trypsin inhibitor stimulation (1). The results presented here as well as those from our previous study (1) indicate that pancreatic enzyme secretion in the rat is subject to feedback inhibition by intestinal proteolytic enzymes (from pancreatic juice). When SBTI is fed or infused, the inhibitor, in effect, removes proteolytic activity from the intestine by binding (as enzyme–inhibitor complexes) intestinal trypsin and chymotrypsin irreversibly, as well as by decreasing the activation of chymotrypsinogen. The effect of

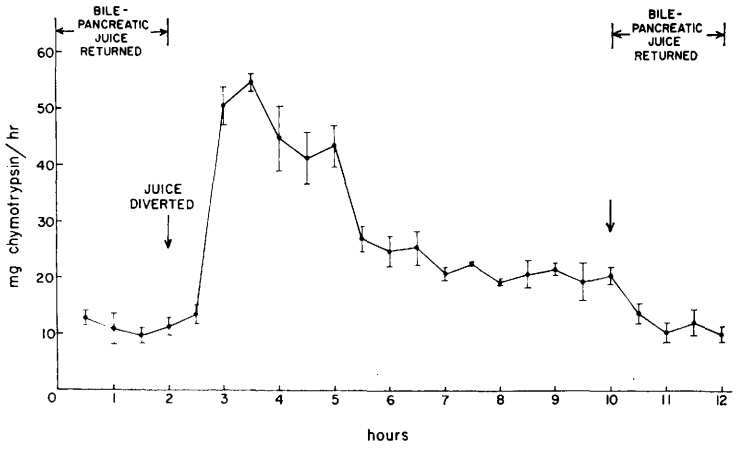

FIG. 5. Effect of diverting bile-pancreatic juice from the intestine on pancreatic enzyme secretion. Bile-pancreatic secretions were not returned to the intestine for an 8-hr period. Pancreatic enzyme secretion is expressed as the total chymotrypsin activity of the bile-pancreatic juice. Each point is the mean ± SEM from 3 animals.

SBTI is therefore similar to the effect of diverting bile-pancreatic juice, that is, they both remove the feedback inhibition by trypsin and chymotrypsin and a large, apparently maximal, output of pancreatic enzymes is produced (Figs. 4 and 5).

We propose that protein (or proteinaceous substrate) also reduces feedback inhibition from trypsin and chymotrypsin in the intestine by forming enzyme–substrate complexes with these enzymes, resulting in an increase in pancreatic enzyme secretion.[2] However, in contrast to the SBTI–enzyme complex, the protein–enzyme complex is transient, and the enzymes become "free" again as the protein is digested. Thus, according to this proposal, increased secretion of pancreatic enzymes would continue only as long as it was needed to digest protein (or proteinaceous substrate). When the enzymes reached a level that exceeded their substrate, they would be free to exert their feedback inhibition and pancreatic secretion would return to basal.

Since prehydrolyzed protein would not complex with the enzymes, protein hydrolysates would not be effective stimulants for pancreatic enzyme secretion as was shown in anesthetized animals for hydrolysates of soy protein (Fig. 1) or in conscious animals with casein hydrolysates (Figs. 2, 3). In studies in progress, partially hydrolyzed casein (*e.g.*, Sheffield Pepticase, 35% αN/TN) evoked a smaller secretory response than casein, but a larger response than the more extensively hydrolyzed casein preparations used in the studies reported here. In the present experiments enzymatically hydrolyzed casein (Fig. 3b) evoked a greater response than did the acid hydrolysate (Fig. 3a), which was essentially a mixture of amino acids. This pattern of response would be expected since partially (incompletely) hydrolyzed casein would bind more proteolytic enzyme than extensively hydrolyzed casein, but less enzyme than intact casein.

The results of this study may help explain the phenomenon of pancreatic adaptation to protein in the diet. The pancreas responds to increased protein in the diet by increasing its content of proteolytic enzyme (especially chymotrypsin), and of amylase and lipase as well in some studies (7–9). Trypsin inhibitors in the diet produce qualitatively a similar adaptation, but the effect is much greater than that seen with protein alone (9, 10).

[2] According to this hypothesis, trypsin and chymotrypsin are not effective in suppressing pancreatic enzyme secretion when they are complexed, whether as enzyme–substrate complexes or as enzyme–inhibitor complexes. A possible mechanism by which trypsin and chymotrypsin suppress pancreatic enzyme secretion was discussed previously (1).

Substitution of casein by hydrolyzed casein caused the proteolytic enzyme content of the pancreas to *decrease* (11, 12). The reduction in enzyme occurred even when a 20% casein diet was replaced by one containing 60% hydrolyzed casein. These changes were observed after 2–3 wk on the particular diet studied. In light of the results we have presented, which show that hydrolyzed casein is a very weak stimulant of enzyme *secretion* in contrast to intact casein, it is reasonable to suggest that the secretagogue effect of dietary proteinaceous materials could be an important factor in producing the observed pancreatic adaptive changes. Consistent with this possibility are reports (9, 13) that injections of CCK-PZ (sc) into rats 3 times/day for 1 wk produced pancreatic adaptation similar to that observed with increased dietary protein (or trypsin inhibitor). Therefore, it is not improbable that stimulation of the pancreas by endogenous CCK-PZ, released by protein or trypsin inhibitor in the intestine, is the event which leads to pancreatic adaptation. If this is the case, one would expect a direct relationship to exist between the secretagogue activity of a proteinaceous foodstuff and its ability to produce changes in pancreatic size and enzyme content. We are presently engaged in experiments to test this hypothesis.

Summary. Pancreatic enzyme secretion was studied in conscious, recovered rats with bile-pancreatic juice collected directly via a cannula. When bile-pancreatic juice was collected and continuously returned to the intestine, introduction of protein evoked a marked increase in enzyme secretion, while protein hydrolysates were not effective stimulants. Diversion of bile-pancreatic juice, or introduction of soybean trypsin inhibitor, resulted in very large increases in enzyme secretion. The results support the concept of negative feedback control of pancreatic enzyme secretion, and it is proposed that protein stimulates increased pancreatic enzyme secretion by reducing feedback inhibition produced by trypsin and chymotrypsin in the intestine.

The authors express their thanks to Raul Reyes for his technical assistance.

1. Green, G. M., and Lyman, R. L., Proc. Soc. Exp. Biol. Med. **140**, 6 (1972).

2. Green, G. M., and Lyman, R. L., Proc. Soc. Exp. Biol. Med. **136**, 649 (1971).

3. Lyman, R. L., Wilcox, S. S., and Monsen, E. R., Amer. J. Physiol. **202**, 1077 (1962).

4. Hummel, B. C. W., Can. J. Biochem. Physiol. **37**, 1393 (1959).

5. Wang, C. C., and Grossman, M. I., Amer. J. Physiol. **164**, 527 (1951).

6. Thomas, J. E., "The External Secretions of the Pancreas," Chap. V. Thomas, Springfield, IL (1950).

7. Desnuelle, P., Reboud, J. P., and Abdeljlil, A., "Ciba Symposium: The Exocrine Pancreas," Churchill, London (1962).

8. Grossman, M. I., Greengard, H., and Ivy, A. C., Amer. J. Physiol. **138**, 676 (1942).

9. Snook, J. T., J. Nutr. **97**, 286 (1969).

10. Geratz, J. D., and Hurt, J. P., Amer. J. Physiol. **219**, 705 (1970).

11. Grossman, M. I., Greengard, H., and Ivy, A. C., Amer. J. Physiol. **141**, 38 (1944).

12. Yudkin, J., and Howard, F., Brit. J. Nutr. **17**, 281 (1963).

13. Rothmann, S. S., and Wells, H., Amer. J. Physiol. **213**, 215 (1967).

Received Dec. 1, 1972. P.S.E.B.M., 1973, Vol. 142.