# A Protein Less Sensitive to Trypsin, Guanidinated Casein, Is a Potent Stimulator of Exocrine Pancreas in Rats (43950)

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*Abstract.* **Previously, we have shown that, in rats that have had bile-pancreatic juice (BPJ) diverted from the proximal small intestine for 7 days, the exocrine pancreatic secretion was enhanced after they were fed a casein, fat-free diet. This demonstrates that the pancreatic secretion is stimulated by dietary protein with a pancreatic protease-independent pathway. To examine the chemical structure of casein responsible for the enhancement of pancreatic secretion, we prepared chemically modified casein in which lysine residues were guanidinated. Secretion of protein, amylase, and chymotrypsin in the chronic BPJ-diverted rat was increased much more after the rats were fed a diet containing guanidinated casein (250 g/kg diet) than after they were fed a diet containing intact casein (250 g/kg diet). In normal rats whose diverted BPJ was returned to the duodenum, the increases in the pancreatic secretion after consuming the guanidinated casein diet were comparable to those after consuming the intact casein diet. *In vitro* digestibility of guanidinated casein by trypsin and chymotrypsin was much lower than that of intact casein. Also, guanidinated casein inhibited tryptic hydrolysis of benzoyl-L-arginine *p*-nitroanilide to a lesser extent than did intact casein as determined by an *in vitro* assay. These results demonstrate that guanidinated casein is less sensitive to trypsin than is intact casein and that the structure that is sensitive to trypsin is not involved in the stimulation of pancreatic secretion in diverted rats. The results evidence that masking luminal trypsin activity does not predominantly contribute to the enhancement of pancreatic secretion in 7-day BPJ-diverted rats. Also, in normal rats, the luminal protease-independent mechanism may play a role partly in increasing the pancreatic secretion by dietary protein.** [P.S.E.B.M. 1995, Vol 210]

Enhancement of pancreatic secretion by dietary protein in rats is associated with masking luminal pancreatic protease activity, especially trypsin, in the proximal small intestine, which constitutes negative feedback regulation (1, 2). This regulatory mechanism is also known in pigs (3). We previously demonstrated that the pancreatic secretion was enhanced by feeding a 25% casein, fat-free diet to rats with 7-day bile-pancreatic juice (BPJ) diversion from the proximal small intestine (4). The finding reveals that the pancreatic secretion in the diverted rat is increased by a luminal protease-independent mechanism because the pancreatic proteases are absent from the proximal small intestine. In BPJ-diverted rats, the pancreatic secretion was not increased after they were fed a low-protein diet (5), and gastric acid was not associated with the increases in the pancreatic secretion (6). These results suggest that dietary casein itself evokes the increases in the pancreatic secretion of the chronic BPJ-diverted rat.

The purpose of the present study was to examine the chemical structure of casein which is responsible for enhancing the pancreatic secretion in the 7-day

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BPJ-diverted rat. Chemical structures of dietary protein associated with the negative feedback regulation are trypsin-sensitive sequences, which contain lysine and arginine residues. So, we prepared a chemically modified casein in which lysine residue is converted to homoarginine by guanidination, and observed the response of the pancreatic secretion to feeding with a diet containing guanidinated casein and intact casein in normal and 7-day BPJ-diverted rats. Also, sensitivity of guanidinated casein to pancreatic proteases was compared with that of intact casein *in vitro*.

## Materials and Methods

**Animals and Diets.** Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan), weighing about 250 g, were operated on after a 24-hr fast for implantation of a bile-pancreatic duct catheter and a small intestinal catheter under pentobarbital anesthesia (sodium pentobarbital 40 mg/kg body wt, Nembutal; Abbott Laboratories, North Chicago, IL). Bile-pancreatic catheter for diversion of BPJ (polyethylene, SP28, i.d. 0.4 mm, o.d. 0.8 mm, Natsume Seisakusyo, Tokyo, Japan) was implanted into the common bile-pancreatic duct and led behind the neck, as described previously (5, 7). A small intestinal catheter for returning BPJ (Silascone No. 00, i.d. 0.5 mm, o.d. 1.0 mm; Dow Corning, Kanagawa, Japan) to the intestine was placed 1 cm proximal from the amupulla of Vater (normal rats) or 45 cm distal from the ligament of Treitz (BPJ-diverted rats). The silastic catheter was led behind the neck subcutaneously and connected to the bile-pancreatic catheter to maintain the BPJ flow. Before suture of the abdominal wall, 1 ml of a chondroitin sulfate solution (1% CHONDORON Inj.; Kaken Pharmaceutical Co. Ltd., Tokyo, Japan) was administered into the peritoneal cavity to prevent intestinal adhesion. In the diverted rats, BPJ flow bypassed the duodenum and jejunum through the catheter, and the BPJ was diverted from the proximal small intestine.

The rats were fasted for 1 day after the operation, then fed a stock diet (Table I) (8–11) for 5 days, and fasted for 1 day before experiments (total diversion period was 7 days). The period is enough to recover from operation, as previously reported (7). After the second 1-day fast, normal and BPJ-diverted rats were given orally 2 g of fat-free test diet containing guanidinated or intact casein (250 g/kg diet) (test diet; Table I) for 30 min. Almost all the given test diet was consumed within 30 min in both the normal and diverted rats. BPJ was collected for 3 min from the connector between the pancreatic and intestinal catheters behind the neck at 30 and 60 min before, and at 30, 60, 90, 120, 150, and 180 min after the feeding, as described previously (5, 7). During this period, BPJ was continuously returned to the intestine throughout the experiment. The experiment using normal rats was performed separately. The rats were kept in a temperature-controlled room at 23° ± 2°C throughout the experiments.

**Table I.** Composition of Diets

| | Stock Diet (g/kg diet) | Casein Test Diet (g/kg diet) | |
|---|---|---|---|
| | | Intact | Guanidinated |
| Intact casein[a] | 250 | 250 | — |
| Guanidinated casein | — | — | 250 |
| Corn oil[b] | 50 | — | — |
| Mineral mixture[c] | 40 | 40 | 40 |
| Vitamin mixture[d] | 10 | 10 | 10 |
| Granulated vitamin E[e] | 1.0 | 1.0 | 1.0 |
| Choline bitartrate | 4.0 | 4.0 | 4.0 |
| Sucrose | | To make 1 kg | |

[a] Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
[b] Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to corn oil.
[c] The mineral mixture is prepared based on the AIN-76 Workshop held in 1989 (9). It provided (mg/kg diet); Ca, 4491; P, 2997; K, 3746; Mg, 375; Fe, 100; I, 0.32; Mn, 10.0; Zn, 34.7; Cu, 6.00; Na, 4279; Cl, 6542; Se, 1.05; Mo, 1.00; Cr, 0.50; B, 0.50; V, 0.25; Sn, 2.00; As, 1.00; Si, 20.0; Ni, 1.00; F, 2.72; Co, 0.20.
[d] The vitamin mixture was prepared in accordance with the AIN-76 mixture (10) except that menadione and L-ascorbic acid were added to make 5.81 μmol/kg (11) and 284 μmol/kg (12) diet, respectively.
[e] Vitamin E granule (Juvela, Eisai, Co., Tokyo, Japan) supplied 423 μmol all-rac-α-tocopheryl acetate/kg diet.

The study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

In guanidinated casein, lysine residues were converted to homoarginine by guanidination of ε-amino groups in casein with 1-guanyl-3,5-dimethyl pyrazole nitrate (12), which was synthesized from aminoguanidine nitrate and acetylacetone (Wako Pure Chemical Industries, Osaka, Japan). The conversion efficiency of lysine to homoarginine was 98%, which was evaluated with amounts of lysine and homoarginine in guanidinated casein. Sum of both amino acids were comparable with amount of lysine in intact casein. Intact and guanidinated casein were hydrolyzed by 6 *N* HCl (110°C, 24 hr) and analyzed amino acids by HPLC as phenyl thiocarbamyl (PTC) derivatives with phenyl isothiocyanate (Tokyo Kasei Kogyo, Tokyo, Japan) (13). The HPLC was constructed by Mini-Solvent Delivery System M-600 (Waters Assoc., Milford, MA) and a Wakopak WS-PTC column (4.0 × 200 mm, Wako).

***In Vitro* Digestibility of Intact and Guanidinated Casein and Occupation of Trypsin by These Proteins.** *In vitro* digestibility of intact and guanidinated casein was evaluated at a concentration of 1 g protein/l with purified trypsin (90,000 U/l, EC 3.4.21.4, from bovine pancreas, Type 1; Sigma Chemical Co., St.

Louis, MO) and purified chymotrypsin (2,900 U/l, EC 3.4.21.1, from bovine pancreas, Type 2; Sigma) in Tris buffer, pH 7.8 and 8.1, respectively. During incubation of medium containing a protein and protease at 37°C, increase in amino group by protease hydrolysis was measured photometrically using 2,4,6-trinitrobenzen sulfonic acid sodium salt (Wako) (14).

Masking trypsin activity with intact and guanidinated casein was estimated *in vitro* by inhibition of hydrolysis of benzoyl-L-arginine *p*-nitroanilide (0.62 m*M*, Peptide Institute Inc. Osaka, Japan) by these protein sources in Tris buffer pH 8.1 containing purified trypsin (40,000 U/l, Type 1; Sigma) at 37°C for 10 min, based on an assay method of trypsin inhibition (16). Hydrolysis of benzoyl-L-arginine *p*-nitroanilide was evaluate as the increase in 420 nm absorbance caused by liberated *p*-nitroanilide. Increases in benzoyl-L-arginine *p*-nitroanilide hydrolysis was linear up to 80,000 U/l trypsin.

**Analyses.** Activities of trypsin, chymotrypsin, and amylase were measured in BPJ diluted adequately with saline containing 0.1% Triton X-100. Zymogens of trypsin and chymotrypsin were activated by purified enterokinase (Sigma). Trypsin (17), chymotrypsin (18), and amylase (19) activities were estimated photometrically using synthetic substrates, Nα-p-toluenesulfonyl-L-arginine methyl ester (TAME), *N*-benzoyl-L-tyrosine ethyl ester (BTEE), and procion yellow starch, respectively. Protein content was quantified by modified Lowry's method (20, 21).

**Calculation and Statistical Analyses.** One unit of trypsin and chymotrypsin is defined as the activity that hydrolyzes 1 μmole of substrate/min at 30°C. The activity of amylase is standardized by purified α-amylase from the porcine pancreas (Type 1A, Sigma) at 37°C. Values of secretion rate in the fasting state are presented as the amount of protein or enzyme activities of BPJ secreted for 3 min, and the average of two collections before a test diet feeding (Table II). The values after feeding (Figs. 1 and 2) represent relative rates of secretion in fasting state (secretion of 0 min was 1) because the secretions in the fasting state in the BPJ-diverted rats were much higher than those in the normal rats. Data was analyzed by two-way analysis of variance (ANOVA, diversion and diet in Table II, and time and diet in Figs. 1 and 2). Significant differences among means were determined by the least significant difference ($P < 0.05$).

## Results

Table II shows the secretion rate in the fasting state of normal and BPJ-diverted rats. Amylase secretion in the diverted group was similar to that in the normal group, but protein secretion was 2-fold, and trypsin and chemotrypsin secretion was 3-fold higher in diverted rats than in normal rats in the fasting state, respectively (*P* values of two-way ANOVA for diversion were 0.0003, <0.0001, and <0.0001, respectively).

Figure 1 shows changes in pancreatic protein, amylase, and chymotrypsin secretion after normal rats were fed a test diet containing intact or guanidinated casein (250 g/kg diet). All indications were significantly increased after feeding with the test diets. The secretion reached peak values 30 min after these rats consumed an intact casein diet and 60 min after they consumed a guanidinated casein diet. The protein secretion in guanidinated casein group was very similar to that in the intact casein group. Amylase and chymotrypsin secretion was also not different between both the protein groups except the chymotrypsin secretion 150 min after feeding.

Figure 2 shows changes in pancreatic secretion in chronic BPJ-diverted rats after feeding with an intact or modified casein diet. In the diverted group, the secretion of protein, amylase, and chymotrypsin after feeding with the guanidinated casein diet was much

**Table II.** Secretions of Protein and Enzymes into Bile-Pancreatic Juice in Fasting State of Intact Casein and Guanidinated Casein Groups of Normal and Chronic BPJ-Diverted Rats

| | Protein (mg/3 min) | Amylase (U/3 min) | Trypsinogen[a] (TAME U/3 min) | Chymotrypsinogen[a] (BTEE U/3 min) |
|---|---|---|---|---|
| Normal rats | | | | |
| Intact casein | 0.98 ± 0.11 | 410 ± 58 | 11.1 ± 2.3 | 18.2 ± 2.6 |
| Guanidinated casein | 1.33 ± 0.08 | 426 ± 96 | 12.6 ± 1.9 | 13.9 ± 1.6 |
| Diverted rat | | | | |
| Intact casein | 1.87 ± 0.22 | 354 ± 66 | 33.8 ± 5.6 | 44.1 ± 6.0 |
| Guanidinated casein | 2.18 ± 0.24 | 399 ± 67 | 40.1 ± 4.6 | 52.6 ± 7.2 |
| ANOVA *P* value | | | | |
| Diversion | 0.0003 | 0.5883 | <0.0001 | <0.0001 |
| Diet | 0.1316 | 0.6898 | 0.4353 | 0.7422 |

*Note.* Values are the amount of protein or enzyme units released from the pancreas for 3 min and are the average of two collections before feeding of a test diet. Values represent mean ± SEM for six rats (intact casein group) or seven rats (guanidinated casein group) in normal group, and for 11 rats in BPJ-diverted group (Diverted rat).

[a] Trypsinogen and chymotrypsinogen were measured as trypsin and chymotrypsin activities after activation by enterokinase. Details were described in Materials and Methods.

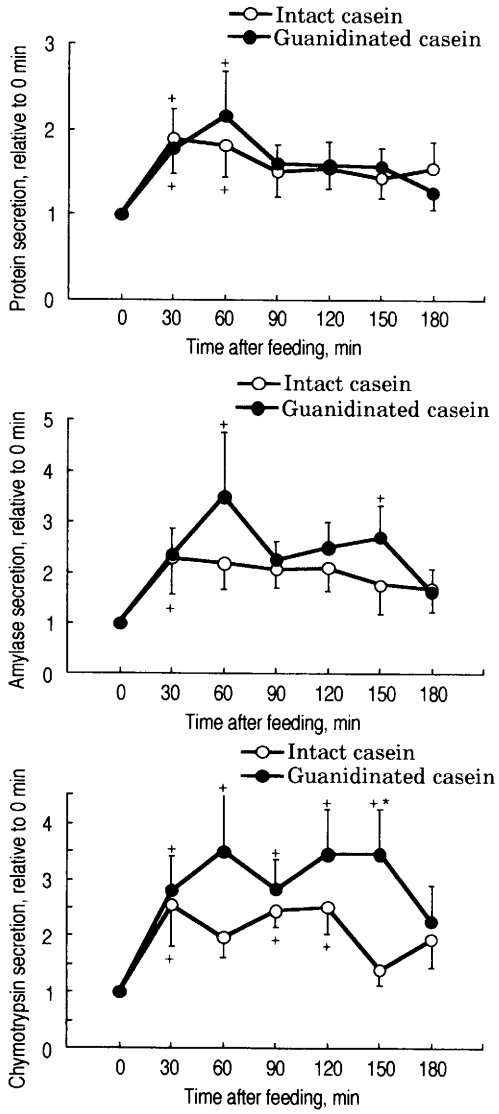


**Figure 1.** The profiles of the pancreatic secretions by feeding with intact or guanidinated casein after a 24-hr fast in normal rats. The values after feeding are multiple of baseline secretion (0 min) in each diet group, which were mean ± SEM ($n$ = 6 in the intact casein group and $n$ = 7 in guanidinated casein group). From the results of two-way analysis of variance, $P$ values of diet were 0.8428, 0.1926, and 0.0170, and of time were 0.0295, 0.0562, and 0.0262 for protein, amylase, and chymotrypsin secretion, respectively. Plus signs represent a significant difference from the value of 0 min in each group ($P < 0.05$). Asterisk represent a significant difference within the same time after feeding ($P < 0.05$).

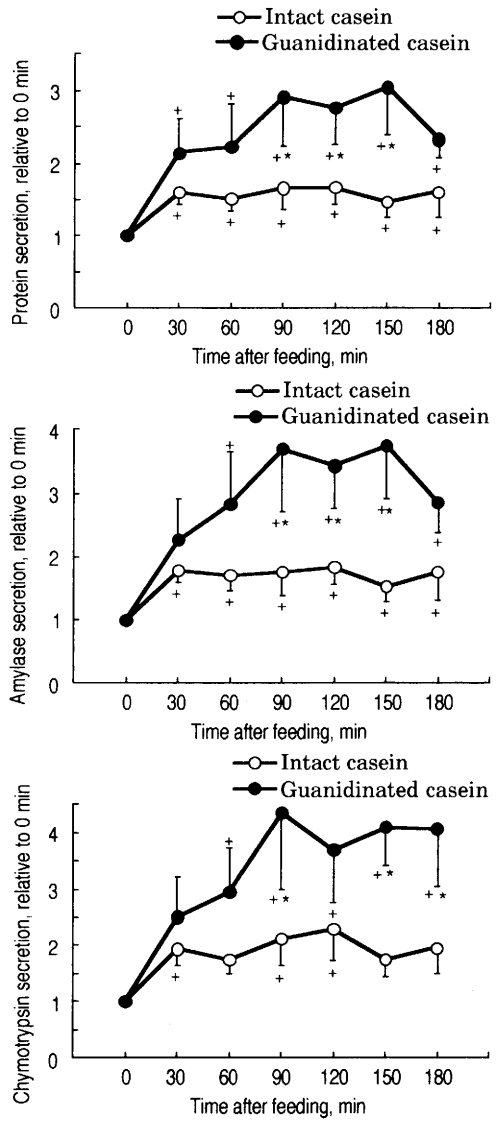


**Figure 2.** The profiles of the pancreatic secretions by feeding intact or guanidinated casein after a 24-hr fast in rats with chronic diversion of bile-pancreatic juice from the proximal small intestine. The values after feeding are multiple of baseline secretion (0 min) in each diet group, which were mean ± SEM ($n$ = 11). From the results of two-way analysis of variance, diet influenced all indications significantly ($P < 0.0001$), and $P$ values of time were 0.0192, 0.0251, and 0.0188 for protein, amylase, and chymotrypsin secretion, respectively. Plus signs represent a significant difference from the value of 0 min in each group ($P < 0.05$). Asterisks represent a significant difference within the same time after feeding ($P < 0.05$).

higher than that after feeding with the intact casein diet. The secretion after feeding with guanidinated casein was increased 3- to 4-fold compared with that in the fasting state (0 min). The secretion after feeding with the intact casein was only 1.5- to 2-fold higher than that in the fasting state, which was similar to that in normal rats fed the intact casein diet. Trypsin secretion in normal and diverted rats was very similar to chymotrypsin secretion (data not shown).

Figure 3 shows *in vitro* digestibility of intact and guanidinated casein by purified trypsin and chymotrypsin. The initial rate of tryptic digestion in both the proteins (time to reach plateau levels) was the same, but the digestibility of intact casein 240 min after incubation was about 3-fold higher than that of guanidinated casein. Digestibility of intact casein by chymotrypsin was also 3-fold higher than that of guanidinated casein.

As shown in Figure 4, masking trypsin activity with guanidinated casein was much less than that with intact casein *in vitro*. That is, guanidinated casein (at a concentration of 2.2 g/l) inhibited hydrolysis of ben-

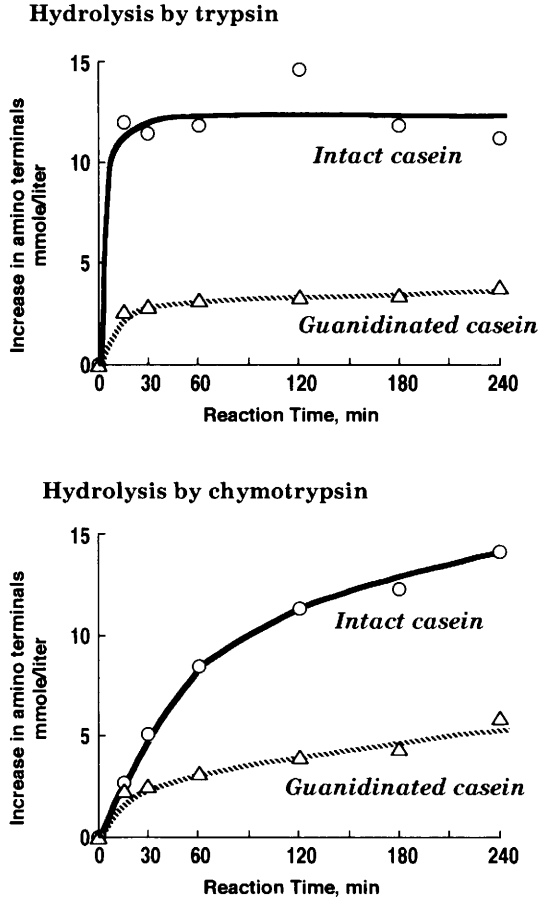


**Figure 3.** *In vitro* digestibility of intact and guanidinated casein with purified trypsin and chymotrypsin. Medium containing the protein source (1 g/l) and purified trypsin (10 mg [90,000 U]/l) or chymotrypsin (50 mg [2,900 U]/l) were incubated at 37°C in Tris buffer, pH 8.1 or pH 7.8, respectively. The value in each point was average of two assays.

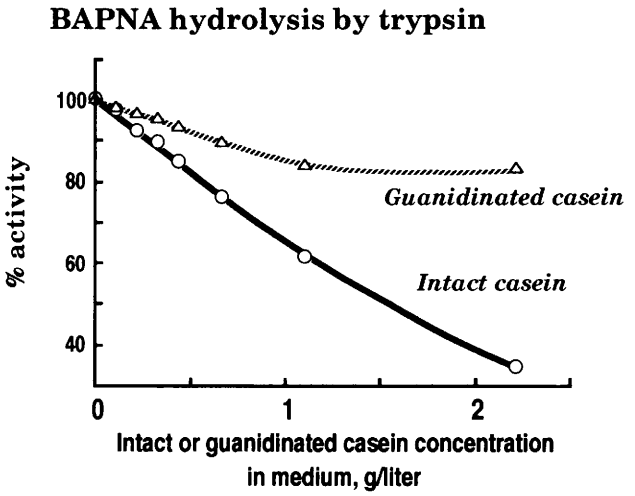


**Figure 4.** Inhibition of tryptic hydrolysis of benzoyl-L-arginine *p*-nitroanilide (BAPNA, masking of trypsin activity) by intact or guanidinated casein at various concentration of these protein sources in Tris buffer, pH 8.1, at 37°C for 10 min. Each point was relative value (% activity) when hydrolytic rate without dietary protein sources are 100%. The value in each point was average of three assays.

zoyl-L-arginine *p*-nitroanilide with purified trypsin to 83% when the hydrolytic rate without guanidinated casein was expressed as 100%. Intact casein at the same concentration as guanidinated casein inhibited the substrate hydrolysis to less than 40%.

## Discussion

We previously reported that pancreatic protein and enzyme secretion was enhanced after feeding 25% protein diets in 7-day BPJ-diverted rats. The increment of the pancreatic secretion in the BPJ-diverted rat does not depend on the mechanism involved in the musking luminal protease activity in the proximal small intestine, because BPJ was completely eliminated from this part of the intestine (4). In the present study, the pancreatic secretion was significantly increased after feeding the diverted rats a test diet containing intact casein. The increment of the secretion was comparable to that in normal rats (Fig. 1 and 2). That is, the luminal protease-independent increase in the pancreatic secretion was shown again in the present study. Furthermore, increases in the pancreatic secretion after feeding a diet containing guanidinated casein was much higher than that after feeding intact casein only in the diverted rats.

A reason that the pancreatic enzyme secretion was higher in rats fed guanidinated casein than in rats fed intact casein is unknown. The *in vitro* digestibility of guanidinated casein by trypsin and chymotrypsin was much lower than that of intact casein (Fig. 3). This result does not agree with that of a report finding that increases in the pancreatic secretion by dietary proteins in normal rats is positively correlated with the digestibility of protein (22), which suggests that the pancreatic secretion in the diverted rats was enhanced with a different mechanism from that in the normal rats. In the BPJ-independent mechanism, protein itself or peptides derived from dietary proteins possibly stimulate directly the release of pancreatic secretagogues from the small intestine. Luminal peptides (CCK-releasing peptides) secretion from the pancreas (23) and the small intestine (24, 25) are known to stimulate CCK secretion directly and enhance the pancreatic secretion. Homologous peptides with the CCK-releasing peptide or other type of active peptides are possibly produced from dietary protein in the small intestinal lumen. Cuber *et al.* (26) reported that cholecystokinin is released by dietary peptides with the luminal protease-independent system. Recently, Beucher *et al.* (27) also showed that glycomacropeptide derived from casein stimulates CCK release from the rat duodeno-jejunum. Modified structure of the guanidinated casein, for example a certine guanidinated structure, may be involved in the stimulatory effect on the pancreatic secretion. The active parts of guanidinated casein (i.e., its low digestibility) may be

retained in the intestinal lumen for rather long period. Guanidinated casein is lacking lysine, so nutritional quality is also low. Relationships between protein quality and the pancreatic secretion, in addition to protein digestibility, should be clarified.

The stimulatory mechanism of the pancreatic secretion in the BPJ-diverted rats is not known. In the negative feedback regulation, enhancement of the pancreatic secretion is involved in the luminal protease activity only in the proximal small intestine (28). In the BPJ-diverted rats used in the present study, the pancreatic secretion in the fasting state was much higher compared with in the normal rats as shown in Table II. Pancreatic enzyme activities in the distal small intestine of the diverted rats may be higher than that in normal rats, whereas the protease activity is absent from the proximal small intestine. Possibly, the luminal pancreatic protease-dependent mechanism for stimulation of pancreatic secretion is induced in the distal small intestine during 7-day BPJ diversion. However, guanidinated casein is a much more potent stimulator than intact casein in the diverted rats (Fig. 2). This finding demonstrates that the luminal protease-dependent mechanism is not responsible for the enhancement of the pancreatic secretion in the BPJ-diverted rats. That is, guanidinated casein is a weaker inhibitor of trypsin than intact casein (Fig. 4), which shows that guanidinated casein is a less sensitive substrate of trypsin than intact casein, and also shows that the masking trypsin activity by guanidinated casein is lower than that by intact casein. The greater increase in the pancreatic secretion caused by feeding guanidinated casein demonstrates that, in 7-day BPJ-diverted rats, the contribution of the luminal protease-dependent mechanism to enhance the pancreatic secretion is small.

In normal rats, the pancreatic enzyme secretion tended to be higher in rats fed guanidinated casein than in rats fed intact casein as shown in Fig. 1. The result suggests that a luminal protease-independent mechanism contributes the increase in the pancreatic secretion also in normal rats, at least partly. In the diverted rats fed guanidinated casein diet, pancreatic secretion was markedly increased in later stages after feeding (90–180 min in Fig. 2). Also, in normal rats fed guanidinated casein, chymotrypsin secretion was significantly higher than in rats fed intact casein 150 min after feeding (Fig. 1). These findings suggest that, in the later stages of feeding, the pancreatic secretion was enhanced with the luminal protease-independent mechanism in both the normal and BPJ-diverted rats.

Recently, Li and Owyang (29), and Soudah *et al.* (30) showed that exogenous and endogenous cholecystokinin stimulate the exocrine pancreatic secretion *via* afferent vagus nerve. Secretin (31) and neurotensin (32) are also involved in the enhancement of the pancreatic secretion. We observed that cholecystokinin did not totally contribute to the increment of the pancreatic secretion with feeding intact and guanidinated casein in the chronic BPJ-diverted rat (unpublished data). These results suggest that dietary proteins or peptides stimulate other than cholecystokinin pathways (for example, direct stimulation of vagus nerve or release of secretin and neurotensin) in the BPJ-diverted rats.

In conclusion, guanidinated casein, which is less sensitive to trypsin than intact casein, is a potent stimulator of pancreatic secretion in chronic BPJ-diverted rats. The result demonstrates that masking luminal protease activity by dietary protein, which is the negative feedback mechanism, is not predominantly responsible for the increases in the pancreatic secretion in chronic BPJ-diverted rats. The finding suggests that low-digestible protein possesses high pancreatic stimulatory activity. This regulatory mechanism seems reasonable in terms of adaptation of digestive function to various dietary proteins.


1. Green GM, Olds BA, Matthews G, Lyman RL. Protein, as a regulator of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med **142:**1162–1167, 1973.
2. Green GM, Miyasaka K. Rat pancreatic response to intestinal infusion of intact and hydrolyzed protein. Am J Physiol **245:**G394–G398, 1983.
3. Ihse I, Lilija P. Effect of intestinal amylase and trypsin on pancreatic secretion in the pig. Scand J Gastroenterol **14:**1009–1013, 1979.
4. Hara H, Narakino H, Kiriyama S. Enhancement of pancreatic secretion by dietary protein in rats with chronic diversion of bile-pancreatic juice from the proximal small intestine. Pancreas **9:**275–279, 1994.
5. Hara H, Kiriyama S. Responses of the exocrine pancreatic secretion to spontaneous feeding in rats with bile-pancreatic juice diversion. Proc Soc Exp Biol Med **198:**732–736, 1991.
6. Hara H, Narakino H, Kiriyama S. Gastric acid independent enhancement of exocrine pancreatic secretion by dietary protein in chronic bile-pancreatic juice diverted rats. Pancreas **11:**173–178, 1995.
7. Hara H, Fujibayashi A, Kiriyama S. Pancreatic protease secretion profiles after spontaneous feeding of casein or soybean protein diet to bile-pancreatic duct cannulated rats. J Nutr Biochem **3:**249–254, 1992.
8. Reeves PG. AIN-76 diet: Should we change the formulation? J Nutr **119:**1081–1082, 1989.
9. American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. J Nutr **107:**1340–1348, 1977.
10. American Institute of Nutrition. Second report of the ad hoc Committee on Standards for Nutritional Studies. J Nutr **110:**1726, 1980.
11. Harper AE. Amino acid balance and imbalance. 1. Dietary level of protein and amino acid imbalance. J Nutr **68:**405–418, 1959.
12. Habeeb AFSA. A new reagent for guanidination of proteins. Can J Biochem Physiol **38:**493–501, 1960.
13. Cohen SA, Bidlingmeyer BA, Tarvin TL. PITC derivatives in amino acid analysis. Nature **320:**769–770, 1986.
14. Habeeb AFSA. Determination of free amino groups in proteins by trinitrobenzen sulfonic acid. Anal Biochem **14:**328–336, 1966.

15. Fields R. The measurement of amino groups in protein and peptides. Biochem J **124**:581–590, 1971.
16. Kakade ML, Simons JJ, McGhee JE, Puski G. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem **51**:376–382, 1974.
17. Rick W. Trypsin. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis (2nd English ed.) New York: Academic Press/ Weinheim: Verlag Chemie, Vol. **2**:pp1013–1024, 1976.
18. Rick W. Chymotrypsin. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis (2nd English ed). New York: Academic Press/Weinheim: Verlag Chemie, Vol **2**:pp1006–1012, 1976.
19. Jung DH. Preparation and application of procion yellow starch for amylase assay. Clin Chim Acta **100**:7–11, 1980.
20. Lowry OH, Rosebrough HJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. J Biol Chem **193**:265–275, 1951.
21. Sugawara K. Influence of triton X-100 on protein determination by Lowry procedure. Agric Biol Chem **93**:2429–2430, 1975.
22. Fushiki T, Kajiura H, Fukuoka SI, Kido K, Semba T, Iwai K. Evidence for an intraluminal mediator in rat pancreatic enzyme secretion: Reconstitution of the pancreatic response with dietary protein, trypsin and the monitor peptide. J Nutr **119**:622–627, 1989.
23. Iwai K, Fukuoka S, Fushiki T, Tsujikawa M, Hirose M, Tsunasawa S, Sakiyama F. Purification and sequencing of a trypsin-sensitive cholecystokinin-releasing peptide from rat pancreatic juice. J Biol Chem **262**:8956–8959, 1987.
24. Miyasaka K, Guan D, Liddle R, Green GM. Feedback regulation by trypsin: Evidence for intraluminal CCK-releasing peptide. Am J Physiol **257**:G175–G181, 1989.
25. Li L, Louie D, Owyang C. A cholecystokinin releasing peptide mediates feedback regulation of pancreatic secretion. Am J Physiol **256**:G430–G435, 1989.
26. Cuber JC, Bernard G, Fushiki T, Bernard C, Yamashita R, Sugimoto E, Chayvialle JA. Luminal CCK-releasing factors in the isolated vascularly perfused rat duodenojejunum. Am J Physiol **259**:G191–G197, 1990.
27. Beucher S, Levenez F, Yvon M, Corring T. Effects of gastric digestive products from casein on CCK release by intestinal cell in rat. J Nutr Biochem **5**:578–584, 1994.
28. Schneeman BO, Lyman RL. Factors involved in the intestinal feedback regulation of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med **148**:897–903, 1975.
29. Li Y, Owyang C. Endogenous cholecystokinin stimulates pancreatic enzyme secretion via vagal afferent pathway in rats. Gastoenterology **107**:525–531, 1994.
30. Soudah HC, Lu Y, Hasler WL, Owyang C. Cholecystokinin at physiological levels evokes pancreatic enzyme secretion via a cholinergic pathway. Am J Physiol **263**:G102–G109, 1992.
31. Guan D, Spannagel A, Ohta H, Nakano I, Chey W, Green GM. Role of secretin in basal and fat-stimulated pancreatic secretion in conscious rats. Endocrinology **128**:979–982, 1991.
32. Trimble ER, Shaw C, Bruzzone R, Gjinovci A, Buchanan KD. Direct stimulation of enzyme secretion from rat exocrine pancreas by neurotensin and its naturally occurring fragments. Gastroenterology **92**:699–703, 1987.