ELSEVIER

Induction of pancreatic trypsin by dietary amino acids in rats: Four trypsinogen isozymes and cholecystokinin messenger RNA

Hiroshi Hara, Naoto Hashimoto, Naoki Akatsuka, and Takanori Kasai

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

We previously demonstrated that feeding a diet containing a high level of amino acid mixture simulating casein (AA) induced an increase in pancreatic protease activities in rats. In the present study, this effect of dietary AA was further characterized with three separate experiments. These experiments (1) examined periodic changes in pancreatic and small intestinal trypsin activities after switching from a 20% (a normal nitrogen level) AA diet to a 60% AA (a high nitrogen level) diet; (2) measured the abundance of mRNA for four trypsinogen isozymes and for intestinal cholecystokinin (CCK) and secretin in rats fed 20% and 60% AA diets for 10 days compared with rats fed 20% and 60% casein diets; and (3) measured the abundance of mRNA for four trypsinogen isozymes after chronic administration of CCK. Trypsin activities were gradually increased in both the pancreas and the small intestinal lumen and reached maximum at 5 days after the switch to the 60% AA diet (Exp. 1). This result is evidence that the increase in the protease activity in the pancreas is due to enhancement of pancreatic trypsin production. In experiment 2, pancreatic trypsinogen isozymes I, II, III, and IV mRNA abundance were evaluated by the Northern blotting method using cDNA probes specific for each isozyme mRNA. Abundance of trypsinogen mRNA without trypsinogen I tended to increase in the rats fed the 60% casein diet but tended to decrease in the rats fed the 60% AA diet compared with the respective normal nitrogen level diet groups without significant difference. CCK mRNA abundance in the jejunal mucosa increased as a result of feeding the 60% casein diet, but not the 60% AA diet. Subcutaneous CCK injections (3.5 nmole/kg body weight/day, twice daily, at 8:30 AM and 7:30 PM) for 10 days resulted in increased pancreatic trypsin activity, whereas the changes in mRNA of the four trypsinogen isozymes was similar between the 20% and 60% casein groups but differed between the 20% and 60% AA groups (Exp. 3). These results suggest that CCK is not involved in the induction of pancreatic trypsin that occurs with feeding of a high AA diet and that the mechanism of protease induction by dietary AA is different from that in the case of dietary protein. (J. Nutr. Biochem. 11:52–59, 2000)

Keywords: dietary amino acids; dietary protein; trypsinogen isozyme; cholecystokinin; messenger RNA; rats

Introduction

High protein diets in experimental animals induces pancreatic protease,[1,2] and cholecystokinin (CCK) is responsible for the protease induction by high protein diet.[3–5] Previously, we showed that feeding a high amino acid diet also induced an increase in pancreatic protease content in rats,[6] even though it is reported that dietary protein, but not dietary amino acid, stimulates pancreatic enzyme secretion in rats.[7] It is well known that feeding a high protein diet causes increased production of pancreatic proteases by masking pancreatic protease activities in the small intestinal lumen in rats,[8,9] thereby allowing luminal CCK-releasing factors to survive. In turn, our previous findings revealed that there is a mechanism for induction of pancreatic protease that is independent of protease activities in the

Address correspondence to Dr. Hiroshi Hara, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan.
Received June 29, 1999; accepted October 12, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/00/$–see front matter
PII S0955-2863(99)00071-6

Table 1 Composition of stock, 20% and 60% casein, or amino acid mixture (AA) diets

	Stock diet	20% casein (AA) diet (g/kg diet)	60% casein (AA) diet (g/kg diet)
Casein (AA)*	250	200 (204)	600 (611)
Corn oil†	50	50	50
Mineral mixture‡	40	40	40
Vitamin mixture§	10	10	10
Granulated vitamin E‖	1.0	1.0	1.0
Choline bitartrate	4.0	4.0	4.0
Sucrose		to make 1 kg	

* Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand). In AA diets, casein was replaced with amino acid mixtures shown in *Table 2*.
† Retinyl palmitate (7.66 μmole/kg diet) and ergocalciferol (0.0504 μmole/kg diet) were added to corn oil.
‡ The mineral mixture was prepared based on the AIN-76 Workshop held in 1989.[12] It provided (mg/kg diet): Ca, 4,491; P, 2,997; K, 3,746; Mg, 375; Fe, 100; I, 0.32; Mn, 10.0; Zn, 34.7; Cu, 6.00; Na, 4,279; Cl, 6,542; 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.
§ The vitamin mixture was prepared in accordance with the AIN-76 mixture (AIN 1976) except that menadione and L-ascorbic acid were added to make 5.81 μmole/kg and 284 μmole/kg diet, respectively.
‖ Vitamin E granules (Juvela, Eisai Co., Tokyo, Japan) supplied 423 μmole all-rac-α-tocopheryl acetate per kilogram of diet.

Table 2 Amino acid content of the 20% and 60% amino acid mixture (AA) diets*

Amino acid	20% AA diet (g/kg diet)
L-Arginine-HCl	8.2
L-Histidine-HCl-H_2O	7.0
L-Isoleucine	9.8
L-Leucine	18.0
L-Lysine-HCl	18.4
L-Methionine	5.2
L-Cystine	0.54
L-Phenylalanine	10.1
L-Tyrosine	11.1
L-Threonine	7.1
L-Tryptophan	2.7
L-Valine	11.8
L-Alanine	5.8
L-Aspartic acid	5.2
L-Asparagine	6.8
L-Glutamic acid	18.2
L-Glutamine	21.0
L-Glycine	3.3
L-Proline	23.9
L-Serine	9.6

* All amino acids used were crystallized preparations. The mixture simulated casein. In the 60% AA diet, all amino acids were present in amounts threefold greater than those in the 20% AA diet shown in this table. The 20% and 60% AA diets were prepared as isonitrogenous to the 20% and 60% casein diet, respectively.

lumen, because luminal amino acids cannot mask protease activities. In addition, we suggested that CCK is not involved in the increase in the pancreatic protease that is observed in rats fed a high amino acid diet.[6] However, the mechanism for increasing pancreatic protease by dietary amino acids is not known.

In humans and dogs, amino acids in the small intestinal lumen stimulate exocrine pancreatic secretion.[10,11] These observations indicate that there is a mechanism for regulating such exocrine functions of the pancreas in response to changes in dietary amino acid levels.

The aim of the present study was to further characterize the increase in content of protease in the pancreas that occurs upon feeding a high amino acid diet. First, we examined the daily changes in trypsin activities in both the pancreas and the small intestinal lumen to indicate that the increment of trypsin in the pancreas is due to increased trypsin production, not to suppression of trypsin secretion. For four trypsinogen isozymes and intestinal CCK and secretin, we examined the changes in mRNA abundance in rats fed a high amino acid diet compared with the mRNA abundance in rats fed a high protein diet and in rats administered CCK.

Experimental methods

Animals

Male Wistar-ST rats (Japan SLC Inc., Hamamatsu, Japan), weighing approximately 100 g, were fed a semi-purified stock diet (*Table 1*)[12,13] for 2 days (Exp. 1) or 5 days (Exp. 2 and 3) to acclimate. For preparation of the amino acid-containing diets, an amino acid mixture simulating casein (AA) was used (*Table 2*).

In experiment 1, the rats were fed an AA diet with a normal nitrogen level [204 g (20%) AA mixture/kg diet] for 7 days and then divided into seven groups on the basis of body weight. The rats in one group were sacrificed immediately (0 day). The rats in five groups were given an AA diet with a high nitrogen level [611 g (60%) AA mixture/kg diet] and sacrificed on the 1st, 2nd, 3rd, 5th, and 10th days after the switch from the 20% AA diet to the 60% AA diet. These AA diets were isonitrogenous to the respective casein diets used in experiment 2. The rats in the last group were fed the 20% AA diet for 10 days and then sacrificed. All rats were sacrificed between 10:00 and 11:00 *am* under pentobarbital anesthesia (50 mg/kg body weight; Abbott Laboratories, North Chicago, IL USA). We confirmed that the stomachs of all rats were filled with chyme. The whole pancreas and the small intestine and its contents were removed immediately. The contents of the small intestine were washed out with 20 mL cold saline. The pancreas and the washout solution were frozen in liquid nitrogen and stored at −40°C until subsequent analyses.

In experiment 2, the rats were divided into four groups. Rats in two groups were fed casein diets with either a normal or high nitrogen level [200 g (20%) and 600 g (60%) casein/kg diet, respectively] for 10 days. The rats in the other two groups were fed the AA diets with either the normal or high nitrogen level used in experiment 1.

In experiment 3, the rats were divided into two groups. The rats in one group were administered CCK by subcutaneous injection (3.5 nmole/kg body weight/day, twice daily, at 8:30 AM and 7:30 PM), and the rats in the other group were administered saline (vehicle) in a manner similar to the case

of CCK solution for 10 days. All rats were fed the 20% casein diet used in experiment 2.

A 100-mg segment of pancreatic tissue of the dorsal area (Exp. 2 and 3) and the 10-cm jejunum segment immediately distal from the ligament of Treitz (Exp. 2) were removed under pentobarbital anesthesia (50 mg/kg body weight; Abbott Laboratories) for extraction of RNA. The mucosa was lightly scraped from the jejunal segment using a glass slide. The pancreatic tissue and mucosa were immediately homogenized in Isogen (RNA extraction mixture, Nippon Gene, Tokyo, Japan) using a Polytron homogenizer (KINEMATICA, Amlehnhalde, Switzerland). Quantification of extracted RNA was achieved spectrophotometrically (absorbance at 260 nm). The residual pancreatic tissue was removed, frozen, and stored as in experiment 1.

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

Analyses

Levels of mRNA for four trypsinogen isozymes in total pancreatic RNA and abundance of mRNA for CCK, secretin, and β-actin in polyadenylated [poly(A) +] RNA from the upper (10 cm) jejunal mucosa were quantified by the Northern blotting method by digoxigenin (DIG)-labeled cDNA hybridization.[14,15] Poly (A)+ RNA was extracted from total RNA of the jejunal mucosa using oligo(dT) latex polymer (Oligotex-dT30 super, Takara Suzo Co., Ltd., Tokyo, Japan). Extracted total or poly (A)+ RNA was electrophoresed on a 1% agarose gel and then transferred from the agarose gel to a nylon membrane (Hybond-N, Amersham International plc., Little Chalfort, UK). In the Northern blot analysis, the RNA on the membrane was hybridized with DIG-labeled trypsinogen I, II, III, and IV, CCK, secretin, or β-actin cDNA, and the DIG-labeled hybridized probes were visualized using a DIG-Luminescent-Detection Kit (Boehringer Mannheim, Mannheim, Germany). The intensity of each mRNA band was quantified by exposing the blots to X-ray film and subsequent scanning densitometry (Flying-Spot Scanner, Shimadzu, Kyoto, Japan).

The cDNAs for trypsinogen I, II, III, and IV were the reverse-transcriptase polymerase chain reaction (RT-PCR) products from total RNA of the pancreas; for β-actin they were the RT-PCR products from total RNA of the jejunal mucosa. CCK and secretin cDNAs were prepared by RT-PCR from poly (A)+ RNA of the jejunal mucosa. Trypsinogen I, II, III, and IV and CCK and secretin cDNA probes were labeled by DIG-PCR[14] from the RT-PCR products using Taq polymerase (Gene Taq, Nippon Gene), specific primers, and DIG DNA labeling mixture (Boehringer Mannheim). Other probes were labeled by means of the DIG DNA labeling kit (Boehringer Mannheim). Primers for CCK were as described previously.[16] Secretin cDNA was prepared using a sense primer (position 24–45) and antisense primer (position 428–450) for rat secretin.[17] cDNAs for pancreatic trypsinogens and β-actin were prepared using a sense primer (position 46–68) and antisense primer (position 439–461) for rat trypsinogen I,[18] a sense primer (position 57–76) and antisense primer (position 404–423) for rat trypsinogen II,[18] a sense primer (position 213–732) and antisense primer (position 775–794) for rat trypsinogen III,[19] a sense primer (position 449–468) and antisense primer (position 724–743) for rat trypsinogen IV,[20] and a sense primer (position 31–51) and antisense primer (position 1000–1020) for rat β-actin.[21] Pancreatic ribosomal RNA, blotted onto membranes, was stained with methylene blue solution (0.04%) and quantified by densitometry.

Trypsinogen in freeze-dried pancreas samples was activated by treatment with enterokinase (Sigma Chemical Co., St. Louis, MO USA) at 30°C for 20 minutes in 15 mmole/L Tris buffer (pH 8.1). Trypsin activities of the pancreas and the small intestinal contents were estimated photometrically using the synthetic substrate *N*α-p-toluenesulfonyl-L-arginine methyl ester (TAME).[22] Amylase activity in the pancreas was measured with procion yellow starch.[23] Protein was measured by a modified version of Lowry's method.[24,25] DNA content was measured by the method of Brunk et al.[26] using 4′,6-diamidino-2-phenylindole. The concentration of total RNA was determined colorimetrically by the orcinol method[27] following extraction as described by Fleck and Munro.[28]

Calculations

Trypsin activity was expressed as TAME U/100 g body weight (total activity) and TAME U/mg protein (specific activity) in the whole (Exp. 1) or residual (Exp. 2 and 3) pancreas (more than 90% of the whole pancreas based on wet weight). One unit of trypsin activity was defined as the amount of activity resulting in hydrolysis of 1 μmole substrate/min at 30°C. Procion yellow starch, the substrate for the amylase assay, was calibrated using a purified α-amylase from porcine pancreas (Type 1A, Sigma Chemical Co.) at 37°C. CCK mRNA and secretin mRNA abundance were expressed as a ratio of β-actin mRNA. Pancreatic trypsinogen mRNA was expressed as a ratio of 18s ribosomal RNA, because β-actin mRNA was not detected with the β-actin probe used in intestinal analyses. The data were analyzed by one-way (Exp. 1 and 3) and two-way (Exp. 2) analysis of variance (ANOVA; nitrogen source and nitrogen level). The existence of a significant difference between groups was determined by Duncan's multiple range test[29] ($P < 0.05$; SAS version 6.07, SAS Institute Inc., Cary, NC USA).

Results

In experiment 1, food intake of rats after switching the diet from 20% AA to 60% AA decreased from 9.83 g/day to 6.66 g/day (means, $P < 0.001$, $n = 8$) and gradually increased for 10 days. Means of food intakes in the 20% AA and 60% AA groups sacrificed on the 10th day were 13.0 and 10.5 g/day ($P < 0.001$, $n = 8$) and body weight gain in the 20% AA and 60% AA groups were 6.10 and 5.49 g/day ($P = 0.076$, $n = 8$).

In experiment 2, the *P*-values of food intake and body weight gain by two-way ANOVA were 0.021 and 0.023 for nitrogen source (S), 0.056 and 0.257 for nitrogen level (L), and 0.794 and 0.894 for S × L, respectively. Means of food

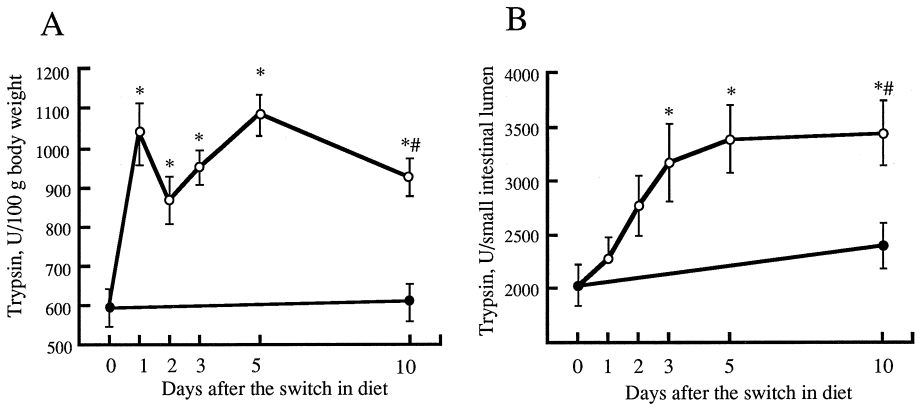

Figure 1 Daily changes in trypsin activities in (*A*) the pancreas and (*B*) the small intestinal lumen after switching from a 20% amino acid diet to a 60% amino acid diet for 10 days (open circle), and the values for rats fed the 20% amino acid diet for more than 10 days (closed circle). Details are described in Materials and methods. Values are the means for eight rats. *P*-values in analysis of variance were <0.001 for pancreatic activity and 0.001 for luminal activity. A mean with an asterisk is significantly different from the value on 0 day ($P < 0.05$), and a mean with a sharp sign is significantly different between the 20% and 60% amino acid diet groups on the 10th day ($P < 0.05$).

intake for the casein and AA groups were 16.2 and 14.7 g/day, respectively ($n = 12$), and means of body weight gain for the casein and AA groups were 7.92 and 6.90 g/day, respectively ($n = 12$), during the 10-day test diet period.

In experiment 3, CCK injections did not influence food intake ($P = 0.583$) or body weight gain ($P = 0.581$). Averages of food intake and body weight gain were 15.1 g/day and 7.32 g/day during the 10-day experimental period.

The trypsin activity (trypsinogen content) of the pancreas markedly increased on the first day after the switch to the 60% AA diet, subsequently decreased on the second day, then increased again, reaching peak values on the 5th day (Exp. 1, *Figure 1A*). The values for all days in rats fed the 60% AA diet were higher than that on day 0. On the 10th day, the activity in the 60% AA group was higher than that in the 20% AA group. The amylase content also increased on the first day, then returned to the initial value on the second day, and was lower on the 10th day than the initial value (data not shown). Luminal trypsin activity gradually increased until the 5th day, then remained at a high value until the 10th day (*Figure 1B*). On the 10th day, the luminal trypsin activity in rats fed the 60% AA diet was higher than that in rats fed the 20% AA diet.

In *Table 3*, pancreatic wet weight and protein, DNA, and RNA content were significantly influenced by nitrogen source and nitrogen level as indicated by the results of two-way ANOVA (Exp. 2). In rats fed the casein diets, all parameter values were significantly greater in the high nitrogen level group than in the normal nitrogen level group. Pancreatic weight and RNA content were higher in the 60% AA diet group than in the 20% AA group. In experiment 3, all parameter values except for DNA content were greater in the CCK-treated group than in the saline-treated group. In the results of two-way ANOVA, all the pancreatic variables were influenced by nitrogen source; that is, these variables were higher in the AA groups than those in the casein groups.

Trypsin activity (trypsinogen content) in the pancreas in the 60% casein and 20% AA groups was higher than that in

Table 3 Pancreatic weight and the protein content, DNA content, and RNA content of the pancreas after feeding 20% and 60% casein or amino acid mixture (AA) diets for 10 days

Diet	Wet weight	Protein content	DNA content	RNA content
		(mg/100 g body weight)		
Exp. 2				
20% Casein	377 ± 16^{c}	62.0 ± 2.6^{b}	5.58 ± 0.368^{b}	8.91 ± 0.382^{c}
60% Casein	451 ± 16^{ab}	89.2 ± 5.0^{a}	7.13 ± 0.410^{a}	11.0 ± 0.305^{b}
20% AA mixture	434 ± 16^{b}	86.5 ± 4.9^{a}	7.10 ± 0.383^{a}	11.0 ± 0.596^{b}
60% AA mixture	498 ± 21^{a}	98.4 ± 5.4^{a}	7.62 ± 0.504^{a}	12.9 ± 0.824^{a}
Two-way ANOVA *P*-values				
Nitrogen source(S)	0.007	0.002	0.023	0.002
Nitrogen level(L)	<0.001	<0.001	0.028	0.002
S × L	0.777	0.113	0.237	0.924
Treatment	Wet weight	Protein content	DNA content	RNA content
		(mg/100 g body weight)		
Exp. 3				
Saline injection	335 ± 13^{b}	65.2 ± 4.3^{b}	4.55 ± 0.404	6.94 ± 0.452^{b}
CCK injection	392 ± 7^{a}	81.2 ± 4.3^{a}	4.37 ± 0.265	8.63 ± 0.464^{a}
ANOVA *P*-values	0.003	0.024	0.714	0.026

All values are mean ± SEM (n = 6). Superscript letters are employed to show significant differences; values in each column not sharing the same letters are significantly different ($P < 0.05$)

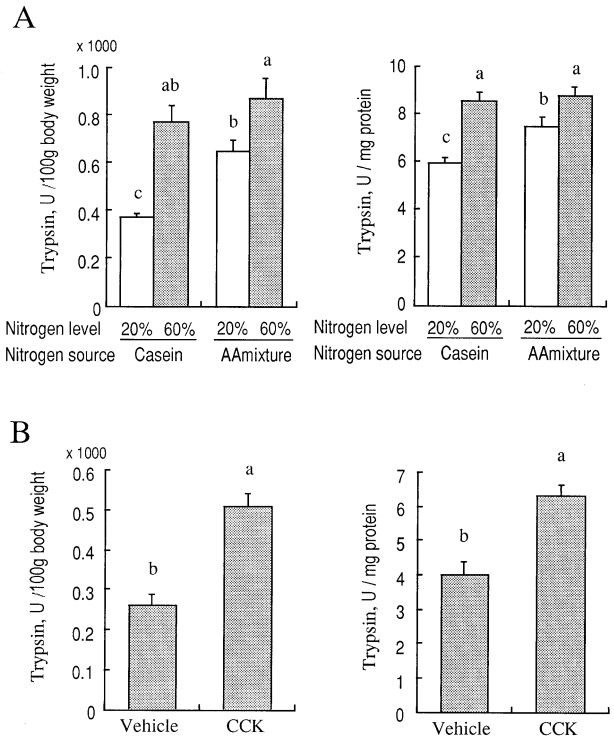

Figure 2 Total and specific activities of trypsin after feeding 60% casein diet and 60% amino acid (AA) mixture diet compared with respective 20% casein and AA diets for 10 days (*A*) and both activities after 10-day injections of cholecystokinin (CCK, 35 nmole/kg body weight/day; *B*). Values are mean with their standard error for six rats. *P*-values estimated by two-way analysis of variance (ANOVA) were 0.005, <0.0001, and 0.146 for nitrogen source (S), level (L), and S × L in total activity, respectively, and were 0.022, <0.001, and 0.073 for nitrogen source, level, and S × L in specific activity, respectively (*A*, Exp. 2). *P*-values estimated by one-way ANOVA were <0.001 in total and specific activities in experiment 3 (*B*). Mean values not sharing a letter are significantly different between diet groups ($P < 0.05$).

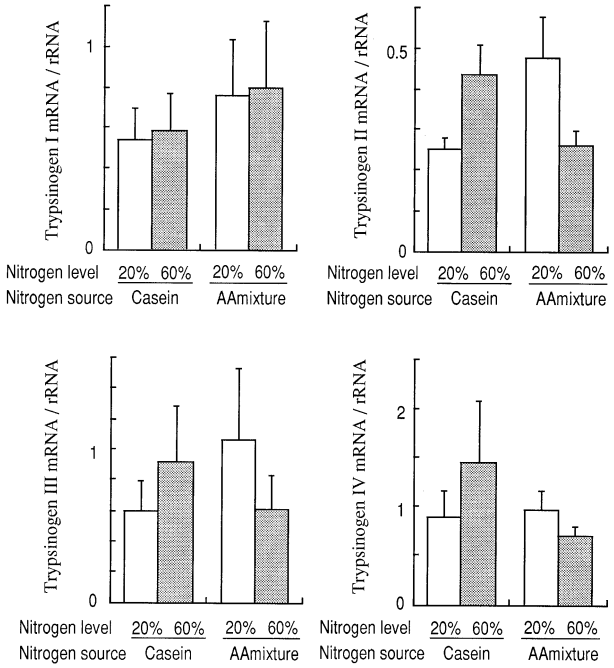

Figure 3 Abundance of pancreatic trypsinogen I, II, III, and IV mRNA trypsin after feeding 60% casein diet and 60% amino acid (AA) mixture diet compared with respective 20% casein and AA diets for 10 days. Values are mean with their standard error for six rats. *P*-values estimated by two-way analysis of variance were 0.382, 0.865, and 0.994 for nitrogen source (S), level (L), and S × L, respectively, in trypsinogen I mRNA; 0.701, 0.809, and 0.008 for nitrogen S, L, and S × L, respectively, in trypsinogen II mRNA; 0.432, 0.820, and 0.040 for nitrogen S, L, and S × L, respectively, in trypsinogen III mRNA; and 0.445, 0.700, and 0.695 for nitrogen S, L, and S × L, respectively, in trypsinogen IV mRNA.

the 20% casein group, and the activity increased as a result of feeding the 60% AA diet compared with rats fed the 20% AA diet (Exp. 2, *Figure 2A*). Changes in trypsin specific activity (U/mg protein) were similar to those observed for the trypsinogen content of the pancreas. Total and specific activities of trypsin in the CCK-treated group were significantly higher than those in the saline-treated group (Exp. 3, *Figure 2B*).

Differences of trypsinogen I, II, III, and IV mRNA abundance (*Figure 3*) were not significant between the diet groups; however, the trypsinogen II, III, and IV mRNA abundance tended to be greater in rats fed the casein diet with the high nitrogen level compared with those fed the casein diet with the normal nitrogen level, but not in rats fed the either of the AA diets.

As shown in *Figure 4*, trypsinogen II mRNA abundance in the CCK-treated group was higher than that in the saline-treated group. The abundance of mRNA for other isozymes did not change with repeated administration of CCK.

CCK mRNA abundance in the jejunal mucosa in rats fed the 60% casein diet was significantly higher than the abundance in the other three groups (*Figure 5*). In contrast, feeding the 60% AA diet did not influence CCK mRNA abundance. Secretin mRNA abundance did not change upon feeding any of the test diets.

Representative Northern blots of pancreatic RNA hybridized with DIG-labeled trypsinogen I, II, III and IV cDNA, and of poly (A)+ RNA from the jejunal mucosa hybridized with CCK, secretin, and β-actin cDNA are shown in *Figure 6* (Exp. 2). Northern blots of pancreatic RNA hybridized with cDNA for trypsinogen I, II, III, and IV are shown in *Figure 7* (Exp. 3).

Discussion

We confirmed the previous finding that trypsin activity (trypsinogen content) in the pancreas was higher in rats fed a 60% AA diet than in rats fed a 20% AA diet (*Figures 1A and 2*). Trypsin activities in the pancreas after the switch from the 20% AA diet to the 60% AA diet were higher than the initial values, and luminal trypsin activity in the absorptive state that reflects trypsin secretion also increased after the switch in diet (*Figure 1*). These findings demonstrate that feeding a high AA diet leads to increases in the amounts of pancreatic protease stored and secreted, indicating that induction of protease production occurred in the pancreas.

In experiment 1, food intake was decreased, and trypsin and amylase activities in the pancreas were markedly increased on the first day after the switch in diet. The value

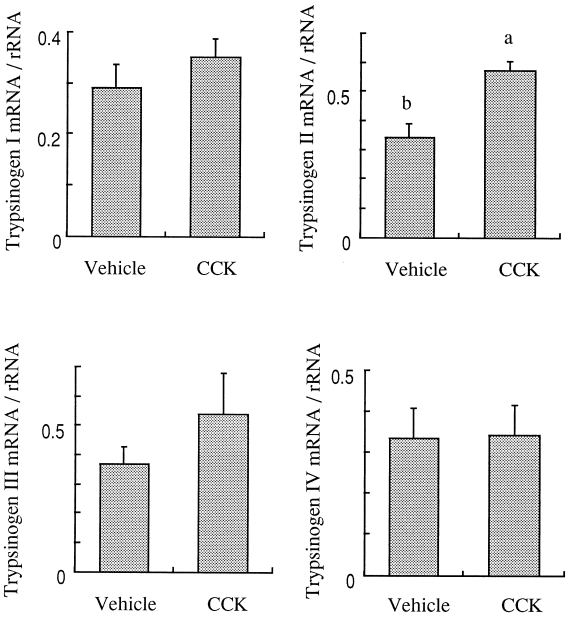

Figure 4 Abundance of pancreatic trypsinogen I, II, III, and IV mRNA after 10-day injections of cholecystokinin (CCK, 35 nmole/kg body weight/day) in comparison with the vehicle (saline) injection group. Values are mean with their standard error for six rats. *P*-values estimated by one-way analysis of variance were 0.330, 0.023, 0.280, and 0.947 for trypsinogen I, II, III, and IV mRNA, respectively. Mean values not sharing a letter are significantly different between diet groups ($P < 0.05$).

of the pancreatic trypsin was decreased on the second day compared with the values on the first day. These results indicate that the marked increases in the pancreatic enzymes on the first day were temporary, and may have been caused by unbalanced rates of secretion and synthesis of these enzymes in the pancreas by suppressing food intake on the first day. The imbalance between the enzyme output and synthesis probably disappeared on the second day because the pancreatic enzymes decreased on the second day. Green et al.[30] also showed that enzyme content in the pancreas was

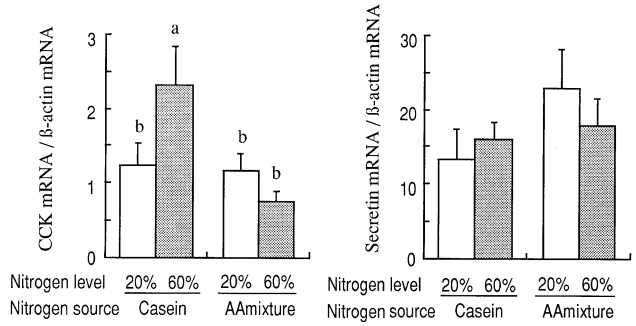

Figure 5 Abundance of cholecystokinin (CCK) and secretin mRNA in the jejunal mucosa after feeding 60% casein diet and 60% amino acid (AA) mixture diet compared with respective 20% casein and AA diets for 10 days. Values are mean with their standard error for six rats. *P*-values estimated by two-way analysis of variance were 0.020, 0.297, and 0.029 for nitrogen source (S), level (L), and S × L, respectively, in CCK mRNA, and 0.170, 0.790, and 0.356 for nitrogen S, L, and S × L, respectively, in secretin mRNA. Mean values not sharing a letter are significantly different between diet groups ($P < 0.05$).

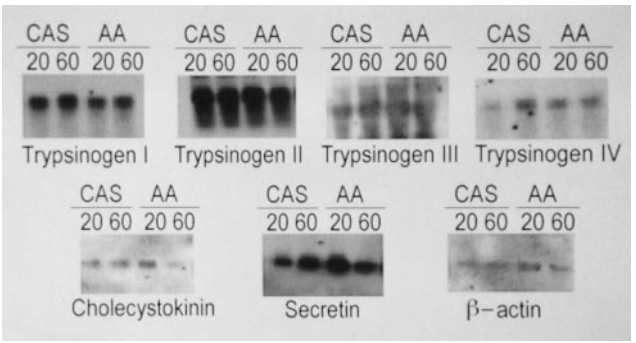

Figure 6 Representative Northern blotting of trypsinogen I, II, III, and IV mRNA in the pancreas, and cholecystokinin, secretin, and β-actin mRNA in the mucosa of the upper (10 cm) jejunal polyadenylated RNA after feeding 20% and 60% casein (CAS) diets and 20% and 60% amino acid mixture (AA) diets for 10 days. Hybridization was performed with digoxigenin-labeled cDNA. RNA separated by a electrophoresis (1% agarose gels) was transferred to nylon membrane and exposed to X-ray film.

temporally increased by suppression of enzyme secretion; however, the increment disappeared within 2 days. These results also show that the temporally increases in enzyme content with suppression of output, if it occurs, may be balanced by changes in enzyme synthesis rate within a few days. Results in *Figure 1* demonstrate that feeding a high AA diet gradually increased the trypsinogen synthesis in the pancreas. The feeding of high AA diet also induced pancreatic growth, as shown in *Table 3*.

In the present study, mRNA abundance of four trypsinogen isozymes was compared between casein and AA as nitrogen sources of diet when rats were fed the diets containing these nitrogen sources with a normal or a high level of nitrogen (*Figure 3*). Trypsinogen II, III, and IV mRNA abundance in the pancreas tended to increase as a result of feeding the high casein diet. Trypsinogen II mRNA abundance in the 60% casein group was significantly higher than that in the 20% casein group (in comparison between casein fed groups with Student's *t*-test, $P < 0.05$). In contrast, trypsinogen II, III, and IV mRNA abundance in the high AA group tended to be decreased compared with those in the normal AA group. These results indicate that the change in profile in mRNA abundance for trypsinogen isozymes upon feeding the high AA diet was different from

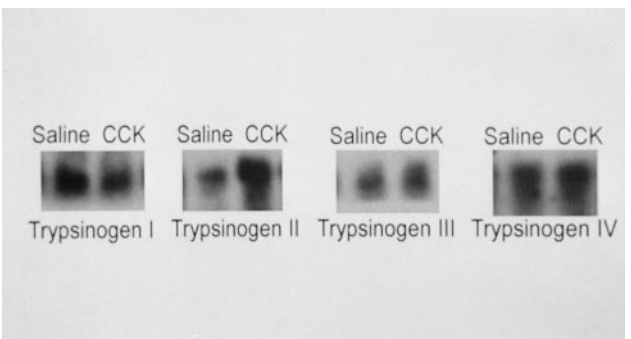

Figure 7 Representative Northern blotting of trypsinogen I, II, III, and IV mRNA in the pancreas after injections of cholecystokinin (CCK) for 10 days.

that in the case of rats fed the high casein diet. There are significant interactions between nitrogen source and nitrogen level (S × L) in trypsinogen II mRNA ($P = 0.008$) and in trypsinogen III mRNA ($P = 0.040$; *Figure 3*). The results of two-way ANOVA provide evidence that changes in mRNA abundance of trypsinogen II and III was not parallel between the casein and AA groups when the level of these nitrogen sources were increased from 20% to 60%. These results reveal that the mechanism of induction of trypsin by feeding a high AA diet differs from that in the case of feeding a high casein diet, and that the control of each trypsinogen isozyme mRNA is not coordinated. Schick et al.[31,32] have reported nonparallel changes in synthesis of trypsinogen isozymes in rats. In addition, the present study showed that the increase in pancreatic trypsin activity that occurs upon feeding high AA diet was not associated with an increase in mRNA abundance for any trypsinogen isozymes. The induction of protease production by feeding the high AA diet may not be due to pretranslational control of trypsinogen synthesis in the pancreas.

Dietary protein induces enhanced production of pancreatic protease,[33] and CCK is involved in such protease induction.[4,5] Dietary protein directly[34] or indirectly stimulates intestinal CCK secretion. In the indirect mechanism, dietary proteins mask luminal proteases, especially trypsin activity, enabling luminal CCK-releasing factors to survive.[35–37] Luminal AA cannot mask protease activities. Dietary AA may stimulate pancreatic acinar cells after intestinal absorption. Another possibility is that luminal or absorbed AA may enhance some hormonal secretion and evoke induction of pancreatic protease via these hormones. Some AA, for example, arginine and leucine, induce the secretion of insulin from the pancreatic islets.[38] In addition, insulin modifies exocrine pancreatic secretion.[39,40] However, the mechanisms of induction of the pancreatic proteases by dietary AA are unknown. Implication of the pancreatic protease induction with feeding a high amino acid diet may be a response for high nitrogen feeding. A high amino acid load may be a signal of feeding high protein diets in the pancreas. Another possible implication is that high dietary amino acid may need more efficient digestion of endogenous luminal protein because the mixture of amino acid is not absorbed in proportion to the composition of the mixture.[41] Absorption of some amino acids, for example, threonine, histidine, and tyrosine, were delayed in rats fed an amino acid mixture. It may be harmful for the body, especially in feeding high amino acid diets. The efficient digestion and absorption of a large pool of luminal endogenous protein may moderate the unbalanced absorption of individual amino acids.

CCK mRNA abundance in the jejunal mucosa increased as a result of feeding a high protein diet (*Figure 5*). This is the first observation that intestinal CCK mRNA is increased by dietary protein in normal rats. In contrast, CCK mRNA abundance did not change upon feeding the high AA diet, but rather the average values tended to be lower compared with the rats fed the 20% AA diet. The change in the profile of mRNA abundance of trypsinogen isozymes after treatment with exogenous CCK was similar to that observed with feeding the high casein diet, but not the high AA diet (*Figures 3 and 4*). We previously showed that a potent CCK-A receptor antagonist, devazepide, does not affect the increase in pancreatic protease that occurs when feeding a high AA diet.[6] Results of the present study strongly support that CCK is not involved in the induction of trypsin and the pancreatic growth that occurs upon feeding a high AA diet.

In conclusion, in rats, production of pancreatic trypsinogen is induced by feeding a high AA diet, and this induction is not associated with any increase in the abundance of mRNA for trypsinogen isozymes, CCK mRNA, or secretin mRNA, in the jejunal mucosa.

References

1 Grossman, M.I., Greengard, H., and Ivy, A.C. (1942). The effect of dietary composition on pancreatic enzymes. *Am. J. Physiol.* **138,** 676–682

2 Wicker, C., Puigserver, A., and Scheele, G. (1984). Dietary regulation of levels of active mRNA coding for amylase and serine protease zymogens in the rat pancreas. *Eur. J. Biochem.* **139,** 381–387

3 Temler, R.S., Dormond, C.A., Simon, E., and Morel, B. (1984). The effect of feeding soybean trypsin inhibitor and repeated injections of cholecystokinin on rat pancreas. *J. Nutr.* **114,** 1083–1091

4 Gasslander, T., Axelson, J., Hakanson, R., Ihse, I., Lilja, I., and Rehfeld, J.F. (1990). Cholecystokinin is responsible for growth of the pancreas after pancreaticobiliary diversion in rats. *Scand. J. Gastroenterol.* **25,** 1060–1065

5 Morisset, J., Guan, D., Jurkowska, G., Rivard, N., and Green, G.M. (1992). Endogenous cholecystokinin, the major factor responsible for dietary protein-induced pancreatic growth. *Pancreas* **7,** 522–529

6 Hara, H., Narakino, H., and Kiriyama, S. (1995). Induction of pancreatic growth and proteases by feeding a high amino acid diet dose not depend on cholecystokinin in rats. *J. Nutr.* **125,** 1143–1149

7 Schneeman, B.O., Chang, I., Smith, L.B., and Lyman, R.L. (1977). Effect of dietary amino acids, casein, and soybean trypsin inhibitor on pancreatic protein secretion in rats. *J. Nutr.* **107,** 281–288

8 Green, G.M., Olds, B.A., Matthews, G., and Lyman, R.L. (1973). Protein, as a regulator of pancreatic enzyme secretion in the rat. *Proc. Soc. Exp. Biol. Med.* **142,** 1162–1167

9 Green, G.M. and Miyasaka, K. (1983). Rat pancreatic response to intestinal infusion of intact and hydrolyzed protein. *Am. J. Physiol.* **245,** G394–G398

10 Thimister, P.W., Hopman, W.P., Sloots, C.E., Rosenbusch, G., Willems, H.L., Trijbels, F.J., and Jansen, J.B. (1996). Role of intraduodenal proteases in plasma cholecystokinin and pancreaticobiliary responses to protein and amino acids. *Gastroenterology* **110,** 567–575

11 Meyer, J.H. and Kelly, G.A. (1976). Canine pancreatic response to intestinally infused protein and protein digests. *Am. J. Physiol.* **231,** 682–691

12 Reeves, P.G. (1989). AIN-76 diet, should we change the formulation? *J. Nutr.* **119,** 1081–1082

13 American Institute of Nutrition. (1977). Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **107,** 1340–1348

14 Seibl, R., Holtke, H.J., Ruger, R., Meindl, A., Zachau, H.G., Rasshofer, R., Roggendorf, M., Wolf, H., Arnold, N., and Wienberg J. (1990). Non-radioactive labeling and detection of nucleic acids. III. Applications of the digoxigenin system. *Biol. Chem. Hoppe Seyler* **371,** 939–951

15 Lion, T. and Haas, O.A. (1990). Nonradioactive labeling of probe with digoxigenin by polymerase chain reaction. *Anal. Biochem.* **188,** 335–337

16 Miyasaka, K. and Funakoshi, A. (1995). Involvement of gene expressions of cholecystokinin and secretin in luminal feedback regulation in conscious rats. *Pancreas* **10,** 200–203

17 Itoh, N., Furuya, T., Ozaki, K., Ohta, M., and Kawasaki, T. (1991). The secretin precursor gene, structure of the cording region and expression in the brain. *J. Biol. Chem.* **266,** 12595–12598

18 MacDonald, R.J., Stary, S.J., and Swift, G.H. (1982). Two similar

but nonallelic rat pancreatic trypsinogens. Nucleotide sequences of the cloned cDNAs. *J. Biol. Chem.* **257,** 9724–9732
19 Lutcke, H., Rausch, U., Vasiloudes, P., Scheele, G.A., and Kern, H.F. (1989). A fourth trypsinogen (p23) in the rat pancreas induced by CCK. *Nucleic Acids Res.* **17,** 6736
20 Fletcher, T.S., Alhadeff, M.A., Craik, C.S., and Largman, C. (1987). Isolation and characterization of a cDNA encoding rat cationic trypsinogen. *Biochemistry* **26,** 3081–3086
21 Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z., and Yaffe, D. (1983). The nucleotide sequence of the rat cytoplasmic beta-actin gene. *Nucleic Acids Res.* **11,** 1759–1771
22 Rick, W. (1974). Trypsin. In *Methods of Enzymatic Analysis*, 2nd English ed., Vol. 2 (H.U. Bergmeyer, ed.), pp. 1013–1024, Academic Press, New York, NY, USA
23 Jung, D.H. (1980). Preparation and application of procion yellow starch for amylase assay. *Clin. Chim. Acta* **100,** 7–11
24 Lowry, O.H., Rosebrough, H.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* **193,** 265–275
25 Sugawara, K. (1975). Influence of triton X-100 on protein determination by Lowry procedure. *Agric. Biol. Chem.* **93,** 2429–2430
26 Brunk, C.F., Jones, K.C., and James, T.W. (1979). Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92,** 497–500
27 Kerr, S.E. and Seraidarian, K. (1945). The separation of purine nucleosides from free purines and the determination of the purines and ribose in these fractions. *J. Biol. Chem.* **161,** 293–303
28 Fleck, A. and Munro, H.N. (1962). The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochim. Biophy. Acta* **55,** 571–583
29 Duncan, D.B. (1995). Multiple range and multiple F tests. *Biometrics* **11,** 1–42
30 Green, G.M., Jurkowska, G., Berube, F.L., Rivard, N., Guan, D., and Morisset, J. (1992). Role of cholecystokinin in induction and maintenance of dietary protein-stimulated pancreatic growth. *Am. J. Physiol.* **262,** G740–G746
31 Shick, J., Verspohl, R., Kern, H., and Scheele, G. (1984). Two distinct adaptive responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. *Am. J. Physiol.* **247,** G611–G616
32 Schick, J., Kern, H., and Scheele, G. (1984). Hormonal stimulation in the exocrine pancreas results in coordinate and anticoordinate regulation of protein synthesis. *J. Cell Biol.* **99,** 1569–1574
33 Giorgi, D., Renaud, W., Bernard, J.-P., and Dagorn, J.-C. (1985). Regulation of proteolytic enzyme activities and mRNA concentrations in rat pancreas by food content. *Biochem. Biophys. Res. Commun.* **127,** 937–942
34 Hara, H., Narakino, H., and Kiriyama, H. (1994). 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
35 Fushiki, T., Kajiura, H., Fukuoka, S.-I., Kido, K., Semba, T., and Iwai, K. (1989). 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
36 Li, L., Louie, D., and Owyang, C. (1989). A cholecystokinin releasing peptide mediates feedback regulation of pancreatic secretion. *Am. J. Physiol.* **256,** G430–G435
37 Miyasaka, K., Guan, D., Liddle, R., and Green, G.M. (1989). Feedback regulation by trypsin, evidence for intraluminal CCK-releasing peptide. *Am. J. Physiol.* **257,** G175–G181
38 Floyd, J.C. Jr., Fajans, S.S., Conn, J.W., Knopf, R.F., and Rull, J. (1966). Stimulation of insulin secretion by amino acids. *J. Clin. Invest.* **45,** 1487–1502
39 Kanno, T. and Saito, A. (1976). The potentiating influences of insulin on pancreozymin-induced hyperpolarization and amylase release in the pancreatic acinar cell. *J. Physiol. (Lond.)* **161,** 505–521
40 Lee, K.Y., Zhou, L., Ren, X.S., Chang, T.-M., and Chey, W.Y. (1990). An important role of endogenous insulin on exocrine pancreatic secretions in rats. *Am. J. Physiol.* **258,** G268–G274
41 Hara, H., Funabiki, R., Iwata, M., and Yamazaki, K. (1984). Portal absorption of small peptides in rats under unrestrained conditions. *J. Nutr.* **114,** 1122–1129