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L-Homoarginine suppresses exocrine pancreas in rats

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Summary. Previously, we found that guanidinated casein, a L-homoarginine-containing protein, was a more potent stimulator of pancreatic enzyme secretion than intact casein in rats. In this study, we examined secretory response and adaptation of the exocrine pancreas to the administration of free L-homoarginine in normal and bile-pancreatic juice (BPJ)-diverted rats. An intraperitoneal injection of L-homoarginine (10 mg/rats) produced immediate and transient reduction in pancreatic secretion in BPJ-diverted rats, but not in normal rats. The BPJ-diverted rats were fed with either a 25% casein, 45% casein, or 45% casein diet supplemented with L-homoarginine (19 g/kg diet) for 4 days. Feeding of a diet containing L-homoarginine inhibited the pancreatic adaptation induced by the high-protein diet. These results indicate that L-homoarginine has an inhibitory effect on the secretion and production of exocrine pancreatic enzyme in BPJ-diverted rats, and L-homoarginine may have an antagonistic effect on CCK receptors.

Keywords: L-Homoarginine – Pancreatic secretion – Pancreatic hypertrophy – Bile-pancreatic juice diversion – Hypercholecystokinemia – Rat

Introduction

L-Homoarginine is a non-essential, a non-proteinaceous amino acid that displays some biochemical and physiological activities. In most of studies on L-homoarginine, this amino acid is used as an inhibitor of alkaline phosphatase (Suzuki, 1994; Tojyo, 1983) or as a substrate for nitric oxide synthase (Chen, 1993a; Hrabak, 1994). Some studies have demonstrated that L-homoarginine inhibits lysine transport in rats and humans (Furesz, 1995; Tews, 1986), or stimulates insulin secretion in rats (Blachier, 1989). However, there have been no reports on the effects of L-homoarginine on the exocrine pancreatic function.

Previously, we studied the effects of a protein containing L-homoarginine residues on rat pancreatic enzyme secretion. In that study, guanidinated casein, a chemically modified casein whose lysine residues were converted to L-homoarginine, stimulated pancreatic enzyme secretion more than intact casein in rats with luminal bile-pancreatic juice (BPJ) diversion (Hara, 1995). This suggests that the L-homoarginine-containing structure in protein is more potent in stimlating pancreatic enzyme secretion. However, it is unclear whether free L-homoarginine, absorbed in the blood circulation, is effective in enhancing pancreatic enzyme secretion. In this study, we first examined the acute effect of parenterally administered L-homoarginine on the exocrine pancreatic secretion in normal and BPJ-diverted rats.

The BPJ-diverted rat is a model for the induction of pancreatic enzyme secretion with hypercholecystokininemia (Chen, 1993b; Chen, 1993c). In this rat, inductions of pancreatic enzyme secretion and enzyme content are mainly mediated by a gut hormone, cholecystokinine (CCK) (Lee, 1986; Miazza, 1987; Hara, 1997). This has been proved by several studies that demonstrated elevated pancreatic enzyme secretion and growth in BPJ-diverted rats were blocked by CCK receptor antagonists (Gasslander, 1990; Nakamura, 1989; Nylander, 1992; Rivard, 1991; Taguchi, 1992; Watanapa, 1991). We previously reported that, in BPJ-diverted rats, feeding of a high-protein diet induced higher pancreatic growth and enzyme production than the feeding of a control diet (Hara, 1998), and that this induction was dependent on the action of CCK on the pancreas (Hara, 2000).

As shown below, experiment 1 revealed that L-homoarginine had a specific effect on the pancreatic

secretion only in BPJ-diverted rats. Thus, we next investigated the chronic effect of dietary L-homoarginine on the exocrine pancreas tissue in BPJ-diverted rats. In the second experiment, BPJ-diverted rats were fed a high-protein diet with or without an L-homoarginine supplement for 4 days, and then the exocrine pancreas tissue was analyzed. In the present study, we found a novel physiological effect of L-homoarginine on the exocrine pancreas (enzyme secretion and production) in BPJ-diverted rats.

Materials and methods

Experiment 1~ Pancreatic enzyme secretion in response to an L-homoarginine solution injected into the peritoneum in normal and BPJ diverted rats

Animal preparation

Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan), aged 8 weeks (200–250 g body weight), were fed with a semipurified 25% casein sucrose-based diet for 5 days. After a 24-hr fast, a common bile-pancreatic duct and an intestinal catheter were implanted under anesthesia (sodium pentobarbital 40 mg/kg body weight; Abbott Laboratories, North Chicago, IL, USA) by a previously described method (Hira, 1999). Briefly, the common bile-pancreatic duct was ligated near its entry into the duodenum and cannulated with the tip of a polyethylene catheter (SP 28; 0.4 mm i.d., 0.8 mm o.d.; Natsume Seisakusyo, Tokyo, Japan) connected to a silicone tubing (Silascon SH No. 00; 0.5 mm i.d., 1.0 mm o.d.; Kaneka Medix Co., Osaka, Japan). A silicone catheter (Silascon SH No. 00), for returning BPJ to the lumen, was placed into the duodenum through the gastric fistula (normal rats) or into the upper ileum (= the middle of the whole small intestine) at 45 cm distal from the ligament of Treitz (BPJ-diverted rats). These catheters were tunneled subcutaneously and connected at the back of the neck. After the operation, the rats were fasted for 1 day, fed with a 25% casein diet for 4 days, and then fasted again the day before the pancreatic secretion experiment described below. In BPJ-diverted rats, BPJ flow bypassed the duodenum and jejunum through the catheter, and the BPJ was diverted from the proximal small intestine throughout the experiment. In normal rats, BPJ flowed into the duodenum through a catheter except during BPJ collection. Rats were kept in a controlled room at 23 ± 2°C, with a 12-hr light-dark cycle (08:00–20:00, light period), and the experiments were performed under unrestrained and unanesthetized conditions. 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.

Pancreatic secretion experiments

Bile-pancreatic juice (BPJ) was collected every 15 or 30 min before and after injection of the test solutions under unrestraint condition, according to a previously described method (Hira, 1999). Briefly, instead of the intestinal catheter, an extension catheter (~40 cm) for BPJ collection was connected to the pancreatic catheter at the behind of the neck at each collection period. Secreted BPJ was collected into 1.5 ml tubes on ice through the extension catheter. The catheter was kept overhead to prevent it from being caught by unrestrained rats in the cage. As an injection control, saline (0.9% NaCl, 0.5 ml/rat, sterilized) was administrated into the peritoneum at −90 min. Then, L-homoarginine (L-homoarginine hydrochloride; Sigma Chemical, St. Louis, MO, USA) solution (10 mg/rat, 0.5 ml/rat) was injected into the peritoneum at 0 min by a bolus injection. L-Homoarginine was dissolved in saline at a concentration of 20 mg/ml. BPJ was collected for 3 min, at 30, 60 and 90 min before, and at 15, 30, 60, 90, 120 and 150 min after the injection of L-homoarginine solution. BPJ was returned into the duodenal (normal rats) or ileal (BPJ-diverted rats) lumen throughout the experiments, except during BPJ collection (3 min at each collection).

Experiment 2~ Exocrine pancreatic adaptation to 4 days-feeding of a high-protein diet containing L-homoarginine in BPJ-diverted rats

Animal preparation and diets

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), weighing about 220 g, were fed with a semipurified sucrose-casein-based diet for 5–7 days for acclimation. After a 24-h fasting, surgery was performed to divert the BPJ to the ileum by transposing a duodenal segment, including the ampulla of Vater, to the ileum under anesthesia by an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt; Abbott Laboratories, North Chicago, IL) (Hara, 2000; Newman, 1986; Williamson, 1979). Briefly, the duodenum 1–2 cm distal from the ampulla of Vater was cut and the proximal cut edge was attached to the lateral opening at the upper ileum (45 cm distal to the ligament of Treitz) by end-to-side anastomosis. Then the duodenum 1–2 cm proximal from the ampulla of Vater was ligated and cut at upper side of the ligation. Thus, a 2–4 cm duodenal segment containing the ampulla of Vater was transposed to the upper ileum. The remaining cut edges of the duodenum were end-to-end anastomosed with each other. After the operation, the rats were fed with a semipurified sucrose-casein-based diet (250 g casein/kg diet, without corn oil) for a recovery period of 14 days. The rats were then divided into 3 groups, and each group was fed with either a 25% casein (control), a 45% casein (high-protein), or a 45% casein containing 1.9% L-homoarginine (high-protein + homoarginine) diet for 4 days (Table 1). The L-homoarginine content (19 g/kg diet) was identical to that in 200 g of

Table 1. Composition of test diets

	25% Casein	45% Casein	+ L-Homoarginine
Casein	250	450	450
L-Homoarginine			19
Mineral Mixture	40	40	40
Vitamin Mixture	10	10	10
Cholin Bitartrate	4.0	4.0	4.0
Sucrose		to make 1 Kg	

Values are grams per kilogram of diet. Casein (ALACID) was obtained from the New Zealand Dairy Board, Wellington, New Zealand. The mineral mixture was prepared on the basis of the formulation established at the AIN-76 Workshop held in 1989 (Reeves PG, 1989). It provided (in mg/kg diet) 4491 Ca, 2997 P, 3746 K, 375 Mg, 100 Fe, 0.32 I, 10.0 Mn, 34.7 Zn, 6.00 Cu, 4279 Na, 6542 Cl, 1.05 Se, 1.00 Mo, 0.50 Cr, 0.50 B, 0.25 V, 2.00 Sn, 1.00 As, 20.0 Si, 1.00 Ni, 2.72 F, and 0.20 Co. The vitamin mixture was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1977), except that menadione and L-ascorbic acid were added to make 5.81 μmol/kg and 284 μmol/kg diet, respectively

the guanidinated casein (Hara, 1995) used in our unpublished study. Rats had free access to the diet and water during acclimation and recovery periods. During the test period, rats in all groups were fed 14 g of the assigned test diet as estimated in a preliminary experiment. The experiments were performed in a room controlled at 23 ± 2°C, with a 12-h light-dark cycle (08:00–20:00 light period). 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.

Analysis

The volume of BPJ was measured gravimetrically: one microliter of BPJ was taken as 1 mg, which is the basis of the measurement of pooled BPJ (100 μl = 100 mg). The pancreas tissue was lyophilized, and then homogenated in saline containing 0.1% Triton X-100. Trypsinogen and chymotrypsinogen in the BPJ and the pancreas homogenate diluted with saline containing 0.1% Triton X-100, were activated by purified enterokinase (Sigma Chemical Co., St. Louis, MO, USA) at 30°C for 20 min in 15 mM Tris buffer (pH 8.1). Trypsin and chymotrypsin activity levels were estimated photometrically using synthetic substrates, *N* α-p-toluene-sulfonyl-L-arginine methyl ester (TAME), and *N*-benzoyl-L-tyrosine ethyl ester (BTEE) (Rick, 1976a; Rick, 1976b), respectively. The protein concentration in BPJ and pancreas was quantified with a modified version of Lowry's method (Lowry, 1951; Sugawara, 1975). The pancreatic DNA content was determined by the method of Brunk using 4′,6-diamidino-2-phenylindole (Brunk, 1979).

Calculation and statistical analysis

One unit of trypsin and chymotrypsin was defined as the activity necessary to hydrolyze 1 μmole of substrate for 1 min at 30°C. In the pancreatic secretion experiment, values for the basal state (−90 min) were calculated as the average of two samplings before the injection of saline. Significant differences among mean values were determined by one-way ANOVA in the pancreatic basal secretion and by the least-significant difference method in the pancreatic secretion experiment, and by Duncan's multiple range test in the pancreatic adaptation experiment ($P < 0.05$, SAS ver. 6.07; SAS Institute Inc., Cary, NC, USA).

Results

Experiment 1

In the pancreatic secretion experiment, the BPJ flowed into the duodenal (normal rats) or ileal (BPJ-diverted rats) lumen through the catheter inserted into the common bile-pancreatic duct for 6 days after the operation. Table 2 shows basal pancreatic secretion parameters in both groups before test agent injection. Although there were no significant differences in BPJ flows (volume) between the two groups, protein secretion, chymotrypsin and trypsin secretions in BPJ-diverted rats were significantly ($P < 0.01$) higher (~2 times) than those in normal rats. This indicates that the BPJ diversion by the cannulation procedure successfully elevated pancreatic basal secretion compared to that in normal rats.

Figure 1 shows time courses of pancreatic secretion parameters before and after a L-homoarginine injection. In order to observe the effect of a bolus injection, saline was injected into the peritoneum at −90 min. There were no significant effects on any of the secretion parameters in either group. However, all parameters in BPJ-diverted rats decreased significantly at 15 min after an intraperitoneal L-homoarginine injection. Values were recovered 60~90 min after the L-homoarginine injection. On the other hand, the injection of L-homoarginine did not affect any of the parameters in normal rats.

Experiment 2

In chronic BPJ-diverted rats whose duodenum was transposed to the upper ileum, there was no significant inter-group difference in total food intake or body weight during the experiment, as shown in Table 3. Pancreatic weight in the 45% casein diet group was significantly higher than that in the 25% casein diet group. In rats fed with a 45% casein diet containing 1.9% L-homoarginine, pancreatic weight was not significantly different from that of the 25% casein diet group, but was significantly lower than that of the 45% casein diet group.

Table 2. Basal pancreatic secretion in normal and BPJ-diverted rats

	Normal rats	BPJ-diverted rats	ANOVA *P* value
BPJ volume (μl/3 min)	73.64 ± 9.22	90.64 ± 6.85	0.1645
Protein (mg/3 min)	1.004 ± 0.197	1.956 ± 0.195	0.0049
Chymotrypsin (U/3 min)	26.30 ± 5.98	58.48 ± 6.46	0.0033
Trypsin (U/3 min)	17.03 ± 3.23	45.26 ± 5.07	0.0005

Values represent the volume, protein content, chymotrypsinogen, and trypsionogen units released from the pancreas for 3 min and are the average of two collections before the injection of saline. Values represent mean ± SEM for 7 rats in each group

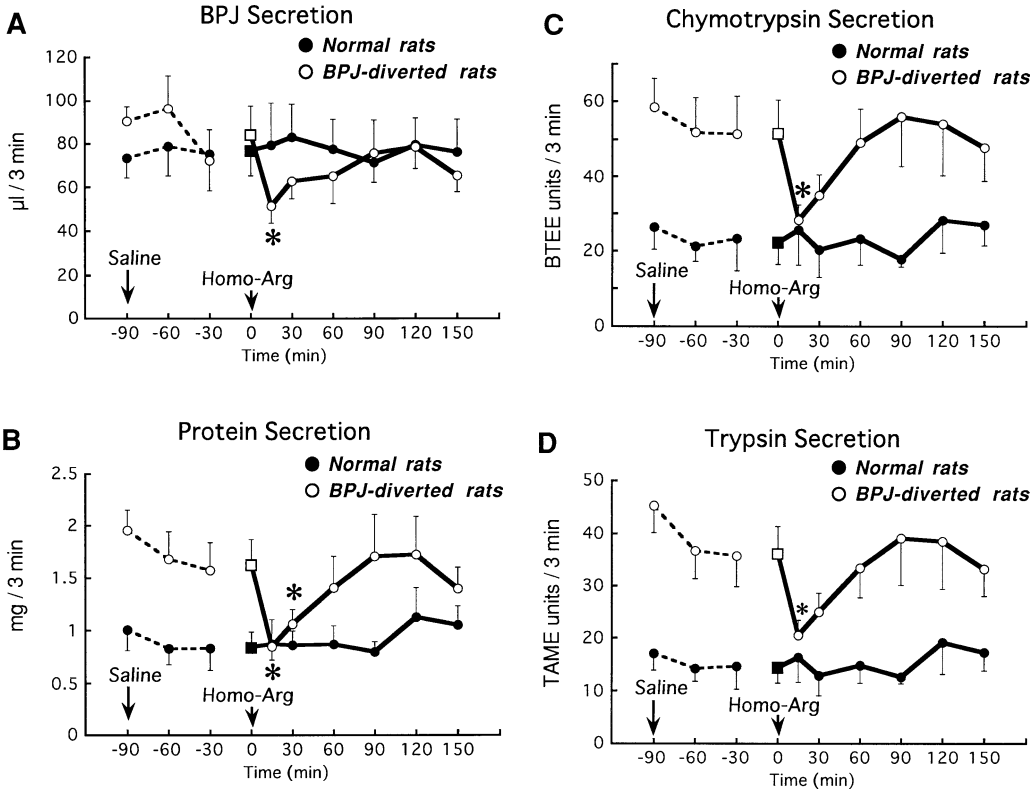

Fig. 1. Pancreatic secretion responses to intraperitoneal injection of saline (at −90 min) and L-homoarginine (at 0 min, 10 mg/rat) in normal rats (closed circles)and BPJ-diverted rats (open circles). **A** Bile-pancreatic juice (*BPJ*) volume, **B** protein content, **C** chymotrypsin activity, **D** trypsin activity in BPJ secreted for 3 min at each time point. Values at −90 min are the average of two collections before a saline injection, and those at 0 min (squares) are the average of two collections after a saline injection, respectively. Values are means ± SEM for 7 rats per group. Asterisks represent a significant difference from the value at −90 min in each group ($P < 0.05$)

Pancreatic protein content in rats fed with the 45% casein diet was slightly but not significantly higher than that in rats fed with the 25% casein diet. However, in rats fed with the L-homoarginine-containing 45% casein diet, protein content was significantly lower than that in rats fed with the 45% casein diet (Fig. 2A). As shown in Fig. 2B, there were no significant differences in pancreatic DNA contents among the three groups. Fig. 2C shows the protein and DNA ratio, an index of pancreatic hypertrophy, which was significantly higher in rats fed with the 45% casein diet than in the other two groups.

Figure 3 shows pancreatic trypsin and chymotrypsin content in BPJ-diverted rats fed with the three different diets. Compared to 25% casein diet group, the content of both proteases were significantly higher in 45% casein diet group, and that in the rats fed with the L-homoarginine-containing diet

Table 3. Growth parameters in BPJ-diverted rats fed with test diets

	25% Casein	45% Casein	45% Casein +Homoarginine	ANOVA *P* value
Food intake (g/4 days)	54.3 ± 0.636	49.7 ± 3.035	54.0 ± 0.809	0.1774
Final body weight (g)	288 ± 4.01	286 ± 5.70	285 ± 6.13	0.9190
Pancreatic dry weight (mg/rat)	489 ± 27.1^{ab}	554 ± 17.8^{a}	451 ± 33.9^{b}	0.0406

Values are means ± SEM of 8 rats in each group. Values not sharing a common superscript letter are significantly different within a column ($P < 0.05$ by Duncan's multiple range test)

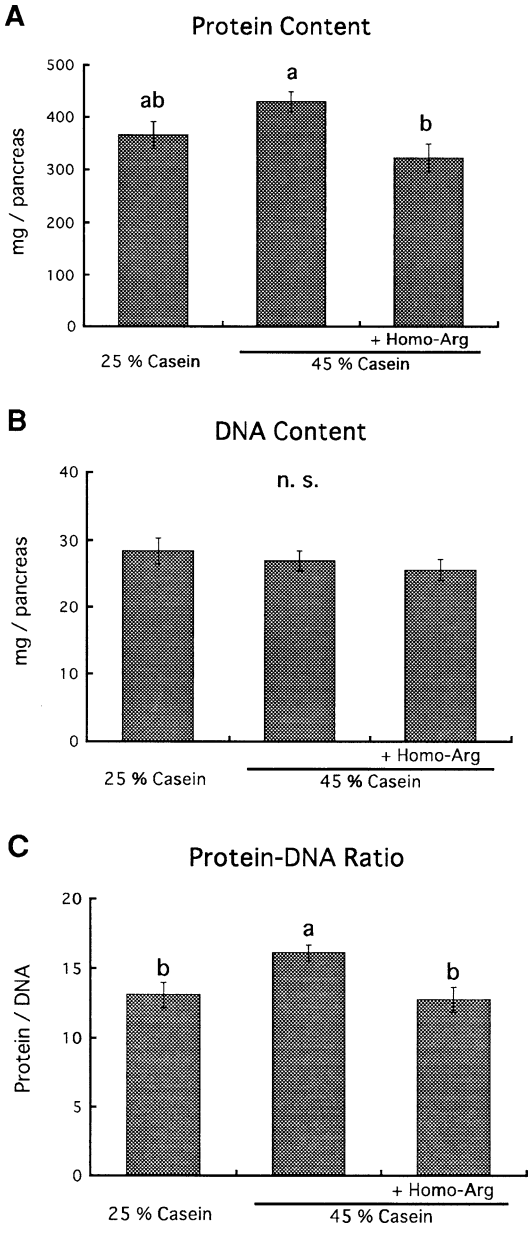

Fig. 2. Pancreatic growth parameters (**A** protein content, **B** DNA content, **C** Protein-DNA ratio) in BPJ-diverted rats fed with a 25% casein diet, a 45% casein diet, or a 45% casein diet containing 1.9% L-homoarginine for 4 days. Values are means ± SEM for 8 rats per group. Mean values not sharing a lower case letter are significantly different between groups ($P < 0.05$). n. s. means not significant

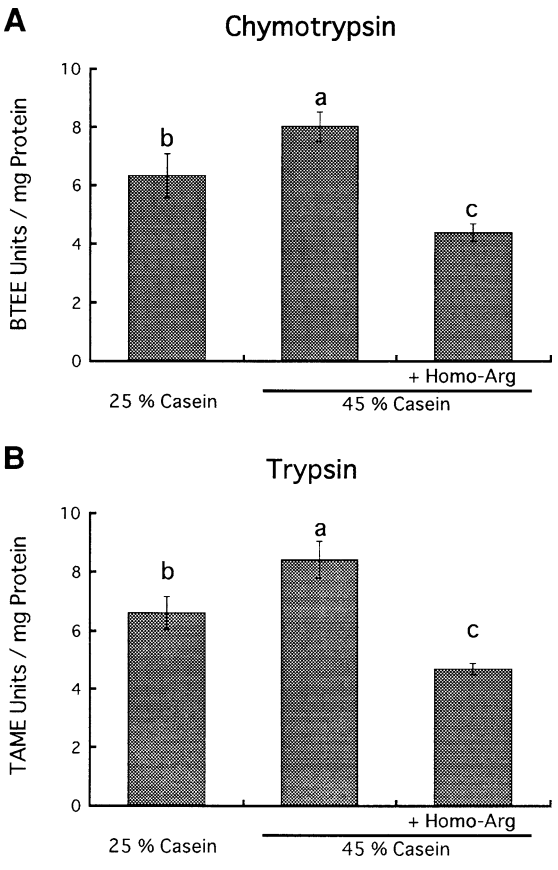

Fig. 3. Pancreatic chymotrypsin (**A**) and trypsin (**B**) activities in BPJ-diverted rats fed with a 25% casein diet, a 45% casein diet, or a 45% casein diet containing 1.9% L-homoarginine for 4 days. Values are means ± SEM for 8 rats per group. Mean values not sharing a lower case letter are significantly different between groups ($P < 0.05$)

were significantly lower than those in the other two groups.

Discussion

As reported in previous papers, L-homoarginine has several biochemical and biological effects in human and rats. 1) (L-Homoarginine inhibits) serum alkaline phosphatases (ALP) but not intestinal ALP (Suzuki, 1994; Tojyo, 1983), 2) is used as a substrate for nitric oxide synthases (Chen, 1993a; Hrabak, 1994), 3) inhibits lysine transport (Furesz, 1995; Tews, 1986), and 4) stimulates insulin secretion from pancreatic beta cells as an L-arginine analogue (Blachier, 1989). However, there has been no report describing the effects of L-homoarginine on the exocrine pancreas *in vivo*. In the present study, we found that L-homoarginine had inhibitory effects on the exocrine pancreas in bile-pancreatic juice diverted rats.

As shown in Fig. 1, L-homoarginine injection into the peritoneum significantly and transiently reduced pancreatic secretion in BPJ-diverted rats, but had no effect in normal rats. Values of protein, trypsin and chymotrypsin secretion in BPJ-diverted rats fell to those in normal rats. A lower BPJ volume at 15 min in

BPJ-diverted rats than in normal rats may be due to the inhibition of pancreatic juice secretion of which relative content in BPJ enhanced by chronic diversion. These results apparently indicate that the effect of L-homoarginine is specific for pancreatic secretion in BPJ-diverted rats, in other words, L-homoarginine inhibits the signal pathway that mediates the elevation of the pancreatic secretion induced by BPJ diversion. In our previous study (Hara, 1995), feeding of guanidinated casein, whose lysine residues were modified to L-homoarginine residues, stimulated pancreatic secretion more than intact casein, in normal and BPJ-diverted rats. And in some papers (Bilski, 1995; Jyotheeswaran, 2000), it was demonstrated that intravenous L-arginine injection stimulated pancreatic secretion in rats via nitric oxide (NO) production. Furthermore, L-homoarginine is reported to be an analogue of L-arginine as a substrate for NO synthase (Chen, 1993a; Hrabak, 1994). Therefore, L-homoarginine was expected to stimulate or enhance pancreatic secretion. By contrary, L-homoarginine had an inhibitory effect on pancreatic secretion enhanced by BPJ-diversion. Since plasma CCK concentrations are raised in BPJ-diverted rats, and pancreatic hypersecretion in this rats is inhibited by CCK receptor antagonists (Folsch, 1987; Nakamura, 1989), CCK is supposed to mediate pancreatic hypersecretion in BPJ-diverted rats. Therefore, it is likely that L-homoarginine has an antagonistic effect on CCK receptors located in the pancreas or the afferent vagal nerve that mediates pancreatic hypersecretion in rats.

As described above, we found L-homoarginine acutely inhibited pancreatic secretion in BPJ-diverted rats. In the next experiment, we examined the effect of feeding a high-protein diet supplemented with L-homoarginine on pancreatic adaptation in BPJ-diverted rats. Because bile-pancreatic catheters should be broken or occluded after more than 10 days in rats and there was no need to collect BPJ (Hara, 2000), we used a transposition procedure in which the duodenal segment containing the Vater papilla was transposed to the upper ileum in order to remove BPJ from the jejunum.

Since it was known that a high-protein diet suppresses food intake, we fed 14 g/day of the diet to each group as estimated from a preliminary study. As a result, there were no significant differences in food intake and body weight among the three groups after the 4-days experimental period (Table 3). At least, in this experimental procedure, an L-homoarginine supplement does not affect growth in rats. As well as in our previous studies (Hara, 1998, 2000), a high-protein diet induced pancreatic growth more than the 25% casein diet. However, a high-protein diet containing L-homoarginine failed to increase pancreatic growth. In the pancreas (Fig. 2), a high-protein diet induced hypertrophy compared with the 25% casein diet, as indicated by a significant increase in the protein content and protein-DNA ratio. L-Homo-arginine also prevented hypertrophy of pancreatic tissue that induced by the 45% casein diet. Further, the L-homoarginine-supplemented 45% casein diet inhibited the 45% casein diet-enhanced induction of pancreatic enzyme content (Fig. 3). These results clearly indicate that L-homoarginine has a suppressive effect on the pancreatic growth and enzyme production induced by a high-protein diet feeding in BPJ-diverted rats, as well as inhibiting the pancreatic enzyme secretion. Using a CCK-A receptor antagonist in BPJ-diverted rats, we previously demonstrated that pancreatic adaptation to a high-protein diet was dependent on CCK (Hara, 2000). Further, some studies demonstrated an involvement of CCK for the pancreatic adaptation to a high-protein diet in normal rats (Green, 1986; Morisset, 1992). On the other hand, a recent paper suggested that CCK is not important for pancreatic adaptation to a high-protein diet using the CCK-deficient mouse (Lacourse, 1999). However, that paper also suggested that the CCK-independent adaptation might be mediated by compensatory mechanisms for the loss of CCK. Therefore, the result in experiment 2 suggests that the addition of L-homoarginine to the diet has an inhibitory effect on CCK-mediated pancreatic adaptation. This supports the hypothesis set up from experiment 1. However, further studies that demonstrate effects of L-homoarginine on plasma CCK, plasma L-homoarginine concentrations, and on the exogenous CCK-stimulated exocrine pancreas are necessary to prove this hypothesis.

In conclusion, we found a novel physiological effect of L-homoarginine, a non-proteinaceous amino acid, on the secretion and adaptation of the exocrine pancreas in rats. In the pancreatic secretion experiment, L-homoarginine injected into the peritoneum reduced pancreatic secretion specifically in BPJ-diverted rats. In the pancreatic adaptation experiment using BPJ-diverted rats, feeding of a high-protein diet supplemented with L-homoarginine inhibited pancreatic hypertrophy and protease

production induced by the feeding of a high-protein diet. These results indicate that L-homoarginine has inhibitory effects on the exocrine pancreas including enzyme secretion and production enhanced by endogenous CCK, and it is likely that L-homoarginine acts as a natural antagonist for CCK receptor(s).

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