Changes in Messenger RNA of Pancreatic Enzymes and Intestinal Cholecystokinin after a 7-Day Bile-pancreatic Juice Diversion from the Proximal Small Intestine in Rats

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We have previously demonstrated the bile-pancreatic juice (BPJ)-independent stimulation of pancreatic enzyme secretion in chronic BPJ-diverted rats. Pancreatic and intestinal adaptation to 7-day BPJ diversion was next examined. Pancreatic enzyme mRNA and cholecystokinin mRNA in the jejunal mucosa were measured in rats with BPJ diverted into the ileum (PBD rats) in comparison with the figures for rats with BPJ returned to the duodenum (normal rats) or laparotomized (Intact) rats under well-nourished conditions. Amylase mRNA in the pancreas was lower and trypsinogen plus chymotrypsinogen mRNA was higher in the PBD rats than in the intact rats. The change in pancreatic mRNA was similar to that in the specific activities of the enzymes after a chronic BPJ diversion. This finding suggests that these pancreatic enzymes were regulated by the mRNA level. The portal concentration of cholecystokinin in the postabsorptive period (exogenously non-stimulated status) was 4-fold higher in the PBD group than in the normal and intact groups. Cholecystokinin mRNA in the jejunal mucosa of PBD rats was somewhat higher than that of intact rats. These results suggest that intestinal cholecystokinin was predominantly increased at the translational or later stage by chronic BPJ diversion.

Key words: pancreatic enzyme; cholecystokinin; messenger RNA; bile-pancreatic juice diversion; rat

The exocrine pancreas adapts to the diet composition and is also modified by endogenous stimulation; for example, bile-pancreatic juice (BPJ) diversion. We have previously demonstrated that dietary protein stimulated pancreatic secretion by a BPJ-independent mechanism in rats with BPJ chronically excluded from the proximal small intestine.1) However, the exocrine pancreatic function is modified by the chronic BPJ diversion. The sensitivity of pancreatic acini to cholecystokinin, but not to carbachol, has been found to be lowered by chronic BPJ diversion, and pancreatic proteases to be markedly induced by chronic diversion.3,4) The pancreatic changes tended to be similar to those with adaptation to a high protein diet,5,6) but adaptation to a chronic BPJ diversion has not been completely characterized.

In many reports, changes in the pancreatic enzymes with BPJ diversion have been evaluated according to enzyme activity (content), but the content was rapidly and markedly changed by secretory stimulation.7) Pancreatic enzyme mRNA may hardly be affected by an acute secretion of enzymes. Cholecystokinin, a potent pancreatic secretagogue, was increased in blood and found to be responsible for pancreatic growth after chronic BPJ diversion.8,9) However, limited information is available about changes in cholecystokinin mRNA in the intestine after a BPJ diversion, cholecystokinin mRNA having been found to be increased after a short-term diversion (4–24 h),10) or partial diversion from the proximal small intestine.11)

The aim of the present study is to examine the changes in pancreatic enzyme and intestinal cholecystokinin mRNA in well-nourished rats with a BPJ diversion for 7 days from the proximal small intestine. The pancreatic growth, enzyme activities, and portal cholecystokinin concentration were also determined in the postabsorptive status. Changes in mRNA levels of pancreatic enzymes and intestinal cholecystokinin have not previously been known in chronic BPJ diverted, well-nourished rats.

Materials and Methods

Animals. Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), were fed on a semi-purified sucrose-based diet containing casein (250 g/kg of diet), corn oil (50 g/kg of diet), and a sufficient amount of minerals and vitamins for 5 days. After a 24-h fast, the rats, weighing 240–260 g, were divided into three groups and anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg of body weight; Abbott Laboratories, North Chicago, IL, U.S.A.). The rats from two groups were operated on to implant a cannula into the common bile-pancreatic duct and the small intestine as described in a previous report.12) Briefly, a polyethylene catheter (SP 26; 0.4 mm i.d., 0.8 mm o.d.; Dow Corning Co., Kanagawa, Japan) for BPJ returning to the intestinal lumen was inserted through a fistula at 1 cm proximal to the ampulla of Vater (normal rat) or of 45 cm distal from the ligament of Treitz to divert BPJ from the proximal small intestine [Pancreatico-biliary-diverted (PBD) rat]. These catheters were led subcutaneously behind the neck and connected to each other to confirm the flow of BPJ during a postoperative period of 7 days. The rats of the third group were subjected to a laparotomy (intact rat). During the postoperative period, each rat had free access to the semi-purified diet, except during the last day when the rats were given 10 g of the diet. This restricted feeding resulted in chyme from the diet being completely absent from the stomach and the proximal small intestine at the time of sacrifice.

A section of pancreas (100 mg of the dorsal area) and the 10-cm jejunum segment immediately distal from the ligament of Treitz were removed for the extraction of RNA after 4 ml of portal blood had been collected in a syringe containing aprotinin and heparin under pentobarbital anesthesia. The pancreas and the mucosa that had been lightly scraped with a slide grass from the jejunum segment were quickly homogenized in an RNA extraction mixture (Isonet, Nippon Gene, Tokyo, Japan) with a Polytron homogenizer (Kinematica, Amiènhaus, Switzerland). The extracted RNA was quantified by measuring the absorbance at 260 nm. After blood

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Abbreviations: BPJ, bile-pancreatic juice; PBD, pancreatico-biliary diversion; DIG, digoxigenin; PCR, polymerase chain reaction.
had been withdrawn from the aorta, residual pancreas was removed and frozen in liquid nitrogen to measure the protein, RNA, DNA, and enzyme activities.

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

**Analyses.** Pancreatic enzyme mRNA was quantified with an *in vitro* translation method by a rabbit reticulocyte lysate system (Amersham, Tokyo, Japan), using L-[35S]-methionine (17.8 MBq/ml) of the assay medium, translation grade, HAS, Hungary) and RNA extracted from the pancreas. The translation products were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 12%), and the radioactivity of the separated products on the gel was directly measured by radio-imaging analysis (BAS-1000 system. Fuji Photo Film Co., Kanagawa, Japan).

Cholecystokinin and β-actin mRNA in the mucosa of the upper (10 cm) jejunal poly(A)+ RNA was quantified by the northern blotting method, using digoxigenin (DIG)-labeled cDNA hybridization.

Poly(A)+ RNA was extracted from total RNA of the jejunal mucosa by oligo(dT) latex polymer (Oligotex-dT30 Super, Takara Shuzo Co., Tokyo, Japan). Extracted poly(A)+ RNA was separated by electrophoresis on 1% agarose gels, and RNA was then transferred from the agarose gel to a nylon membrane (Hybond-N, Amersham, U.K.). The northern blot was hybridized with DIG-labeled cholecystokinin and β-actin cDNA, and the DIG-labeled hybridized probes were detected by using a DIG-luminescence detection kit (Boehringer Mannheim, Mannheim, Germany). The intensity of each mRNA band was quantified by exposing to X-ray film and scanning densitometry (CS9000 Flying-spot scanner, Shimadzu, Kyoto, Japan). The DIG-labeled cholecystokinin cDNA probe was prepared by the reverse-transcribe polymerase chain reaction (RT-PCR) from poly(A)+ RNA of the jejunal mucosa by using the primers previously described and then DIG-PCR from the RT-PCR products by using Taq DNA polymerase (Gene Taq, Nippon Gene), the respective primers and a DIG DNA labeling mixture (Boehringer Mannheim). The cDNA probe for β-actin was an RT-PCR product from total RNA of the jejunal mucosa labeled with a DIG DNA labeling kit (Boehringer Mannheim). The RT-PCR was performed using a sense primer (position 31–51) and antisense primer (position 1000–1020) for rat cytosolic β-actin.

Trypsinogen and chymotrypsinogen in freeze-dried pancreas were activated by enterokinase (Sigma Chemical Co., St. Louis, MO, U.S.A.) at 30°C for 20 min. The trypsin and chymotrypsin activities were evaluated photometrically by using the synthetic substrates, Nα-p-toluenesulfonyl-L-arginine methyl ester (TAME) and N-benzoyl-L-tyrosine ethyl ester (BTEE), respectively. Amylase activity in the pancreas was measured by using porcine yolk starch, and protein was measured by a modification to Lowry's method. DNA concentration was measured by the method of Brunk et al. using 4,6-diamidino-2-phenylindole. The concentration of total RNA was colorimetrically determined by the orcinol method after applying the treatment described by Fleck and Munro.

Plasma cholecystokinin concentration was evaluated by using the bioassay described by Liddle et al. Portal plasma (2 ml) treated with a Sep-Pak C18 cartridge was freeze-dried and incubated with dispersed pancreatic acini prepared from a fasted rat. The amylase released into the medium was quantified, and the cholecystokinin concentration in plasma was calculated by a CCK-8 standard curve.

**Calculations.** The activities of the pancreatic enzymes are presented as U in pancreas (content) and U/mg of protein (specific activity). One unit of trypsin or chymotrypsin is defined as the activity which hydrolyzes 1 μmol of substrate per minute at 30°C. The amylase activity was calibrated against purified a-amylase from porcine pancreas (Type 1A, Sigma Chemical Co.) at 37°C. All data were analyzed by an analysis of variance, and significant differences among groups were determined by Duncan's multiple-range test (SAS version 6.07, SAS Institute Inc., Cary, NC, U.S.A.).

**Results.** Body weight gain during the postoperative period (days 1–6) was unchanged in the PBD rats (4.12 g/day, n = 5) when compared with that of normal (4.27 g/day, n = 7) and intact (4.45 g/day, n = 6, p = 0.9044) rats. All rats bearing a swollen

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBD</td>
<td>1.41 ± 0.13a</td>
<td>14.3 ± 1.03b</td>
<td>32.5 ± 4.26b</td>
<td>291 ± 28.9b</td>
</tr>
<tr>
<td>Normal</td>
<td>0.79 ± 0.04b</td>
<td>9.76 ± 0.64b</td>
<td>12.3 ± 0.90b</td>
<td>151 ± 6.34b</td>
</tr>
<tr>
<td>Intact</td>
<td>0.77 ± 0.06b</td>
<td>9.46 ± 1.07b</td>
<td>15.0 ± 1.10b</td>
<td>176 ± 15.6b</td>
</tr>
</tbody>
</table>

*p Value <0.0001 <0.0001 <0.0001 <0.0001

Each value is mean ± SEM (5 rats for PBD, 7 rats for Normal and 6 rats for Intact).

Values not sharing a common superscript letter differ significantly (p < 0.05).

![Fig. 1. Differences in Pancreatic Amylase, Trypsin, and Chymotrypsin Activities in Rats 7 Days after Bile-pancreatic Juice Diversion into the Jejunum (PBD, 5 Rats), Returned into the Duodenum (Normal, 7 Rats), or Only Laparotomy (Intact, 6 Rats). P Values evaluated by a one-way analysis of variance were 0.1787 for amylase, <0.0001 for trypsin, and <0.0001 for chymotrypsin. Mean values not sharing a letter are significantly different between dietary groups (p < 0.05). NS shows no difference between groups.](image)
bile-pancreatic duct caused by the impairment of BPJ flow were excluded from results.

Table shows changes in the pancreatic weight, DNA, RNA, and protein content 7 days after the BPJ diversion from the proximal small intestine. All indications were about 2-fold higher in the diverted group than in the normal and intact groups. Cannulation of the common bile-pancreatic duct did not influence the pancreatic growth indications (normal vs. intact group). Trypsin and chymotrypsin activities (content) shown in Fig. 1 were clearly higher in the PBD group than in the normal and intact groups, and total amylase activity was not significantly changed 7 days after the BPJ diversion. The specific activities of the two proteases shown in Fig. 2 were significantly higher in the PBD group than in the intact group. That of amylase was significantly decreased by a BPJ diversion.

An imagining photograph of the radioactivity of in vitro translation products (pancreatic enzymes) separated by SDS-PAGE is presented as Fig. 3, while quantitative data

![Image](image_url)

Fig. 3. Imaging Photograph (BAS-1000, Fuji Photo Film Co.) of the Incorporated Radioactivity of L-[³⁵S]-Methionine into in Vitro Translation Products of RNA Extracted from the Pancreas with Rabbit Reticuloocyte Lysate. The translation products were separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 12%).

![Image](image_url)

Fig. 4. Differences in the Relative Amounts of Amylase and Trypsinogen Plus Chymotrypsinogen mRNA in Rats 7 Days after Bile-pancreatic Juice Diversion into the Ileum (PBD, 5 Rats), Returned into the Duodenum (Normal, 7 Rats) or Only Laparotomy (Intact, 6 Rats). p Values evaluated by a one-way analysis of variance were 0.0014 for amylase and 0.0044 for trypsinogen plus chymotrypsinogen mRNA. Mean values not sharing a letter are significantly different between dietary groups (p<0.05).
are shown in Fig. 4. The relative amylase radioactivity (％
of total intensity) was markedly lower in the PBD group
than in the normal and intact groups. Serine protease
(trypsigen + chymotrypsinogen) mRNA was higher in the
PBD group than in the normal and intact groups. Lipase
mRNA tended to be lower in the PBD rats than in the
normal rats, but the difference was not significant (p =
0.1234, data not shown). Carboxypeptidase mRNA was not
changed by PBD (p = 0.4766, data not shown).
Northern blotting of cholecystokinin and β-actin mRNA
(Fig. 5) was quantified by calculating the ratio of
cholecystokinin and β-actin mRNA intensities as shown in
Fig. 6. The cholecystokinin mRNA level in the PBD rats
was significantly higher than in the intact group, but not
in the normal group. Cholecystokinin concentration in
portal blood measured by the bioassay shown in Fig. 6 was
4-fold higher in the PBD rats than in both the normal and
intact groups.

**Discussion**

Exocrine pancreatic adaptation to a chronic BPJ
diversion from the proximal small intestine was evaluated
according to mRNA of the pancreatic enzymes and in-
testinal cholecystokinin in well-nourished rats. The body
weight gain in BPD rats was similar to that in intact rats,
indicating that the rats used in this experiment would have
been of a good nutritional status, because pancreatic
adaptation was impaired by protein malnutrition.

Chronic BPJ diversion from the proximal small intestine
induced pancreatic hypertrophy and hyperplasia (increases
in protein, DNA, and RNA) as shown in the present study,
these results having been previously reported.

The total activities of trypsin and chymotrypsin in the pancreas were
markedly increased, their specific activities being 2-fold
higher in the PBD group than those in the normal and
intact groups (Figs. 1 and 2). Amylase had decreased specific
activity after the chronic BPJ diversion. These results were
observed during a postabsorptive period (non-stimulation
by the diet) and may indicate the synthetic rate of these
pancreatic enzymes. Changes in the specific activities of the
pancreatic enzymes were similar to those of the relative
mRNA content in the pancreas. This agreement suggests
that the induction of pancreatic enzymes after a chronic
BPJ diversion would be involved in the alteration of mRNA
levels.

The relative content of amylase mRNA had decreased 7
days after the BPJ diversion (Figs. 3 and 4), this change
being similar to that after feeding a high-protein diet.
In the present study, amylase mRNA may have been controlled
by activities other than diet, because the diet given to PBD
rats was the same as the diet given to normal and intact
rats, with sucrose as the sole source of carbohydrate. Sucrose
can be digested and absorbed without pancreatic juice. It
is not known why amylase mRNA and specific activity were
decreased by chronic BPJ diversion, in spite of the increase
in that of serine proteases. Protein digestion may be restored
in preference to carbohydrate digestion.

The portal cholecystokinin concentration was 4-fold
higher in the PBD rats when compared with the level in
normal and intact rats. The difference between chronic BPJ
diverted and normal rats has been found to be similar to
observations by Watanapa et al. and Taguchi et al.
A high concentration of portal cholecystokinin during a non-
stimulating stage by diet may reflect a sustained increase in
the synthesis rate of cholecystokinin in the intestine of
chronic BPJ-diverted rats. In the present study, we observed a small increase in jejunal cholecystokinin mRNA by a chronic BPJ diversion (Fig. 6). We could not detect cholecystokinin mRNA in the distal small intestinal mucosa (unpublished data). Thus, the increase in blood cholecystokinin level predominantly depended on translational or later control, because an increase in the postabsorptive state may reflect an enhanced synthetic rate for intestinal cholecystokinin. Miyasaka et al.33 have previously reported that the plasma concentration and intestinal mRNA of cholecystokinin had clearly increased after a 7-day BPJ diversion. In their PBD rats, bile-pancreatic juice was diverted from the proximal and distal small intestine. This result suggests that some factors, that is bile acid or gut hormones, in the distal small intestine play a role in changing the intestinal CCK synthesis.

The results of the present study show that the induction of pancreatic enzymes, especially of serine protease mRNAs, was associated with an increase in cholecystokinin concentration in the blood. The protease induction mediated by cholecystokinin may be associated with an endogenous CCK-releasing peptide produced by the proximal small intestine.34,35 In the lumen of this part of the intestine in chronic BPJ-diverted rats, partly digested protein may be retained because of the absence of pancreatic proteases. Peptides derived from dietary proteins are also possibly involved in the increased portal cholecystokinin concentration and in the induction of pancreatic protease. Beucher et al.36 have shown that a peptic hydrolysate of casein, glycomacropeptide, directly stimulated cholecystokinin released from an isolated vascularly perfused duodenojejunum.

In conclusion, a chronic BPJ diversion from the proximal small intestine resulted in pancreatic protease mRNA being induced and amylase mRNA being depressed under well-nourished conditions; the changes correspond to those in the specific activities of these enzymes in a postabsorptive status. Cholecystokinin mRNA was slightly induced, but a portal concentration of cholecystokinin was markedly increased by the BPJ diversion. The sustained increase in blood cholecystokinin may be mainly involved in the translational or later stage of cholecystokinin synthesis in the intestine.

References
