Absence of Luminal Bile Increases Duodenal Content of Cholecystokinin in Rats (43242)

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Abstract. **The effects of the removal of bile from the proximal intestine on pancreas, plasma cholecystokinin (CCK) concentration, and duodenal content of CCK were examined in rats. Bile was excluded from the duodenum and introduced into the distal ileum through a silastic cannula for 7 days. Pancreatic juice was maintained to be normally secreted into the duodenum. After 7-day bile diversion, plasma CCK concentration and duodenal CCK content were significantly increased in bile-diverted rats. Trypsin content in the proximal intestine in bile-diverted rats was one-half that in control. Pancreatic wet weight, protein content, and DNA content in the pancreas were slightly increased, and lipase content was slightly decreased, by bile diversion, but none of these changes was statistically significant. Amylase content significantly decreased and chymotrypsin content significantly increased in bile-diverted rats. Intragastric administration of camostate (trypsin inhibitor) significantly increased plasma CCK concentration in both bile-diverted and control rats, and the net increase was much greater in bile-diverted rats than in control rats. In conclusion, bile diversion increased duodenal CCK content and increased the CCK response to luminal stimulant.**

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The removal of bile from the intestine has been reported to increase the secretion of pancreatic enzymes in rats (1, 2). The mechanism for the increase in pancreatic secretions by the exclusion of bile has been considered to be due to the reduction by bile and bile acids of the rate of disappearance of trypsin and chymotrypsin activities from the small intestine by some unknown mechanism (1, 3). The decrease in protease activities in the lumen increases pancreatic exocrine secretions (so-called luminal feedback regulation) (4). The increase in pancreatic secretions is mediated by cholecystokinin (CCK) from the intestine (2, 5, 6). However, in recent studies, bile has been reported to regulate pancreatic secretions not only by an indirect effect via luminal proteases, but also by a mechanism(s) independent of luminal protease activities (7, 8). In the previous study (9), we compared the effect of bile flow obstruction with that of bile diversion from the proximal intestine on pancreatic exocrine functions and found that the removal of bile from the intestine (by either bile diversion or bile duct ligation) increased pancreatic secretions. Furthermore, the increases in pancreatic secretions (basal and stimulated by cerulein) in rats with bile diversion were maximal on the seventh postoperative day. Therefore, in the present study, we examined the effects of 7-day bile diversion on pancreatic enzyme contents, plasma CCK, and duodenal CCK concentrations.

Methods

Materials and Chemicals. Camostate (FOY-305). *N,N*-dimethylcarbamoylmethyl-4-(4-guanidinobenzoyloxy)phenylacetate methansulfonate) was a generous gift from Ono Pharmaceutical Co., Osaka, Japan. Synthetic CCK-octapeptide sulfate (CCK-8) was purchased from the Peptide Institute, Osaka, Japan. Bovine serum albumin, soybean trypsin inhibitor (Type I-S), and chromatographically purified collagenase (Type IV) was from the Sigma Chemical Co., St. Louis, MO, and

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minimal Eagle's medium amino acid supplement was from Gibco Laboratories, Grand Island, NY.

Animal Preparations. Male Wistar rats (283–340 g, 13 to 15 weeks old) for bile diversion experiments and female Sprague-Dawley rats (200–250 g) for CCK bioassay were obtained from Shizuoka Jikken Dobutsu (Shizuoka, Japan). Rats were given commercial rat chow (CRF-1, Oriental, Tokyo, Japan) before surgery and during recovery.

Operative procedure. For anesthesia, Enflurane (Abbott Laboratories, North Chicago, IL) was delivered with a vaporizer through a plastic face mask. A midline abdominal incision was made. The common bile duct was ligated below the bifurcation near the liver and a cannula (Silastic Medical Grade Tubing; Dow-Corning, Midland, MI, 0.025 inches. i.d. × 0.047 inches o.d.) was inserted above the ligature. The distal end of this cannula was inserted into the distal ileum. Therefore, bile was excluded from the duodenum and returned to the ileum, and pancreatic juice was secreted into the duodenum. Rats were maintained at 24°C with a 12-hr light/dark cycle and filtered air in the laboratory animal cages (four rats per cage) for 7 days, with free access to food and water. Rats were sacrificed 7 days after the operation. Control rats were sacrificed before the operation. We did not examine sham-operated rats because we had confirmed previously that body weight does not change 7 days after a sham operation, although normal rats of this age gain body weight. Under the anesthesia by ether, 5–7 ml of blood were obtained from the abdominal aorta by a heparinized syringe, and the pancreas and a proximal intestine 20 cm in length were removed. Blood was centrifuged immediately after sampling at 3000 rpm for 15 min at 4°C. Pancreases were weighed and some were submerged in the formaldehyde solution for histologic examination; others were frozen in liquid nitrogen for measuring enzyme and DNA content. The duodenum was washed with 6 ml of cold distilled water, then opened longitudinally. The intestinal mucosa was removed by gentle scraping of the surface with a spatula, and then immediately lyophilized. The intestinal contents were also immediately frozen and lyophilized for measuring trypsin activity. Furthermore, in some of control and bile-diverted animals, 100 mg/kg of camostate was administered through an orogastric tube, and rats were sacrificed 1 hr after the ingestion. Animals were sacrificed between 0900 and 1100 hr without previous fasting.

Assays. *Assay for duodenal CCK.* Since it has been reported that smaller molecular forms of CCK are best extracted at neutral or alkaline pH and CCK 33–39 are mainly extracted in acid, extraction of CCK from the duodenal tissue was performed as reported by Goke *et al.* (10), with some modifications. Briefly, samples were boiled in 2 ml of water for 30 min, homogenized for 2 min, then centrifuged at 300,000*g* for 15 min. The supernatant was assayed in different dilutions. The pellet was then added with 2 ml of 1 *M* acetic acid, allowed to stand for 30 min, and homogenized, and then centrifuged at 300,000*g* for 15 min. The supernatant was assayed in different dilutions. A radioimmunoassay for duodenal CCK was conducted using antiserum OAL-656, ^{125}I-Bolton-Hunter CCK-33, and CCK-8 as a tracer and a standard, respectively (11–13). The antiserum specifically reacted with an amino-terminal region of CCK-8, and bound 100% of both CCK-8 and CCK-33 and 85% of CCK-39, but did not cross-react with the nonsulfated form of CCK-8, gastrin 17-1, or cerulein. The sensitivity of the assay was 1.3 fmol/tube, which is equivalent to 6.6 p*M* as CCK-8. Total CCK concentrations of the tissue samples were estimated as the sum of values from water and acetic acid extracts.

Gel filtration of duodenal mucosa. Duodenal extracts (acid or water extracts) were applied to a Sephadex G-50 superfine column (1.5 × 100 cm), equilibrated, and eluted with 1 *M* acetic acid at a flow rate of 10 ml/hr. Fractions of 2 ml were collected, lyophilized, and reconstituted in 0.5 ml of assay buffer for CCK-radioimmunoassay. The column was calibrated with blue dextran, ^{125}I-insulin, CCK-33, CCK-8, and [^{125}I]Na.

Enzyme and DNA concentrations in the pancreas. The protein concentration was determined according to the method of Lowry *et al.* (14). Chymotrypsin activity was assayed by spectrophotometric methods (15, 16), using *N*-benzoyl-L-tyrosine ethyl ester as a substrate and purified bovine chymotrypsin as a standard (Worthington Biochem, Freehold, NJ) (17, 18). Amylase activity and lipase and DNA content were assayed as described previously (17, 18).

Trypsin activities in the proximal intestine. Trypsin activity was measured, using a substrate tosyl-L-arginine methyl ester, by the spectrophotometric method (15). Samples of lyophilized intestinal contents were homogenized at 0°C in water (2 ml total volume), and a 50- to 200-μl sample was used for assay (19, 20).

Assay for plasma CCK. Plasma CCK concentrations were measured by a bioassay using dispersed acini, as described by Liddle *et al.* (21). A 2- to 3-ml plasma sample was obtained from each rat, and CCK was extracted from these plasma samples by adsorption onto Sep-Pak catridges, washed with 20 ml of water, eluted with 1 ml of acetonitrile:water (1:1, v/v) into a polyethylene scintillation vial, and dried with a flow of nitrogen at 45°C (5). Although 1 ml of plasma was extracted for the measurement of camostate-stimulated CCK concentration, two 3-ml or three 2-ml samples were combined into a 6-ml sample and used for measuring the basal CCK concentration. Acini suspensions (1-ml aliquots) were added to the vial containing the

plasma extracts or various concentrations of CCK-8 and incubated for 30 min at 37°C. Amylase release into the medium and total acinar amylase contents were measured. Values were compared with values of a standard curve of CCK-8, and results were expressed as CCK-8 equivalents.

Statistics. Values were expressed as the mean ± SE. Results were analyzed by Student's t test or two-way analysis of variance. A value of $p < 0.05$ was considered significant.

Results

Body weight was slightly decreased by bile diversion (Fig. 1). Pancreas wet weight and the ratio of pancreas to body weight were slightly higher in rats with bile diversion than in control rats, but differences were not statistically significant (Table I).

CCK in the Duodenal Mucosa. CCK content in the duodenum measured after the extraction by acetic acid was significantly higher in rats with bile diversion than that in the control rats, whereas CCK concentrations extracted by H_2O were not significantly different. Consequently, total CCK content in the duodenum significantly increased in rats with bile diversion (Table II). The gel filtration pattern of control duodenal extract revealed the presence of three CCK immunoreactive peaks. The first peak was observed before the elusion of CCK-33. The second and third peaks were eluted in the same areas as those of CCK-33 and CCK-8, respectively (Fig. 2, upper panel). In contrast, after bile diversion, gel chromatography of duodenal acid and water extracts showed three major peaks (Fig. 2, lower panel), which coincided with three peaks in the upper panel. The first two peaks of immunoreactivity (especially that corresponding to CCK-33) were significantly higher than corresponding peaks in the upper panel of the figure (Fig. 2, lower panel).

Pancreatic Enzyme and DNA Content. Pancreatic wet weight and protein content were slightly higher in rats with bile diversion, but differences between the two groups were not statistically significant (Table I). On the other hand, amylase concentration and content were significantly lower and trypsin concentration and content were significantly higher in bile-diverted rats (Table I). Lipase and DNA content were not significantly different between the two groups.

Table I. Effect of Bile Diversion on Pancreas[a]

	Control rats	Bile-diverted rats
Pancreatic wet weight (mg)	870.0 ± 50.5	931.3 ± 42.0
Pancreas/body ratio	0.29 ± 0.02	0.36 ± 0.01
Protein concentration (mg/g wet pancreas)	281.0 ± 38.5	290.1 ± 12.2
Protein content (mg/whole pancreas)	245.6 ± 38.8	270.0 ± 15.8
Amylase concentration (mg/g wet pancreas)	53.0 ± 4.8[b]	26.0 ± 4.4
Amylase content (mg/whole pancreas)	45.6 ± 3.2[b]	24.7 ± 5.0
Chymotrypsin concentration (mg/g wet pancreas)	11.5 ± 2.8	16.7 ± 1.2[b]
Chymotrypsin content (mg/whole pancreas)	10.4 ± 3.1	15.6 ± 1.5[b]
Lipase concentration (IU/g wet pancreas)	144.8 ± 27.7	87.5 ± 9.4
Lipase content (IU/whole pancreas)	130.1 ± 29.3	82.4 ± 11.6
DNA content (mg/whole pancreas)	5.3 ± 0.9	5.7 ± 0.6

[a] Values are mean ± SE; $n = 5$ for each group.
[b] Indicates a significant difference by Student's t test.

Table II. Duodenal CCK Content and Luminal Trypsin Activities[a]

	Control rats	Bile-diverted rats
Trypsin in the proximal intestine (μg)	76.3 ± 12.4[b]	35.4 ± 3.0
Total CCK content in the proximal intestine (pmol)	31.5 ± 2.9	55.5 ± 8.4[b]
CCK extracted by H_2O (pmol)	25.3 ± 2.1	34.8 ± 6.4
CCK extracted by acetic acid (pmol)	6.6 ± 1.0	19.6 ± 1.9[b]

[a] Values are mean ± SE.
[b] Indicates significant increase by Student's t test; $n = 8$ for each group.

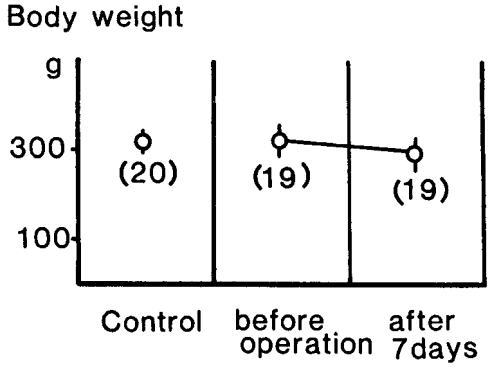

Figure 1. Changes in body weight after the operation. Body weight slightly decreased by bile diversion, but the difference was not statistically significant. Numbers in parentheses are numbers of rats.

Plasma CCK Concentrations. Changes in plasma CCK-like bioactivities before and after camostate ingestion are shown in Figure 3. There were significant effects by both treatments of bile diversion and camostate administration when analyzed by two-way analysis of variance (Fig. 3). The plasma CCK concentration after bile diversion was slightly, but significantly, higher than the value in control group (0.45 vs 1.39 pM).

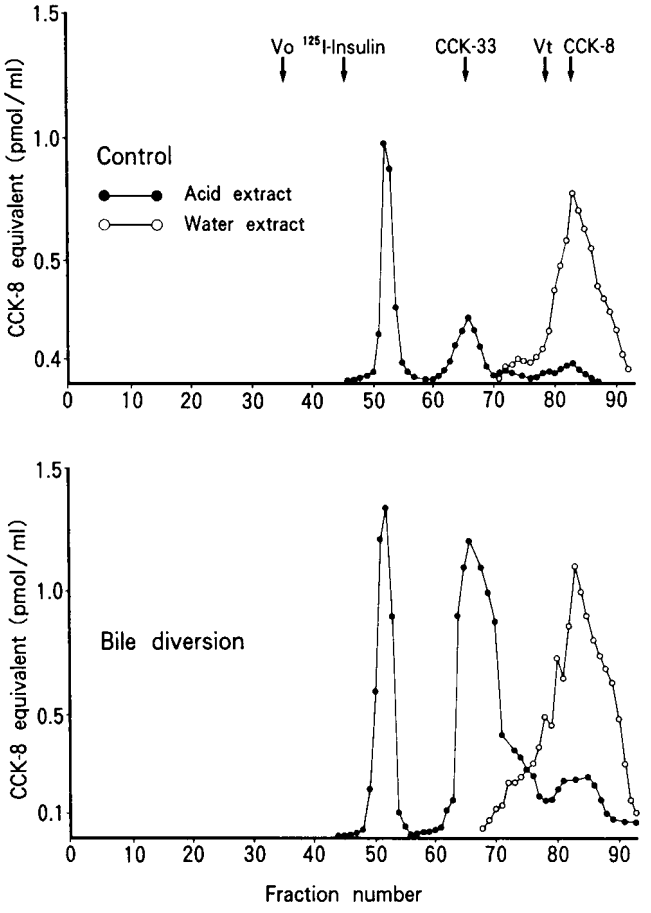

Figure 2. Upper panel: Gel filtration of duodenal CCK in control rats. CCK-8, 33, and probably 58 were eluted. Lower panel: All fractions increased in rats with 7-day bile diversion, and the increase of acid extract was statistically significant. A representative figure in each group is shown.

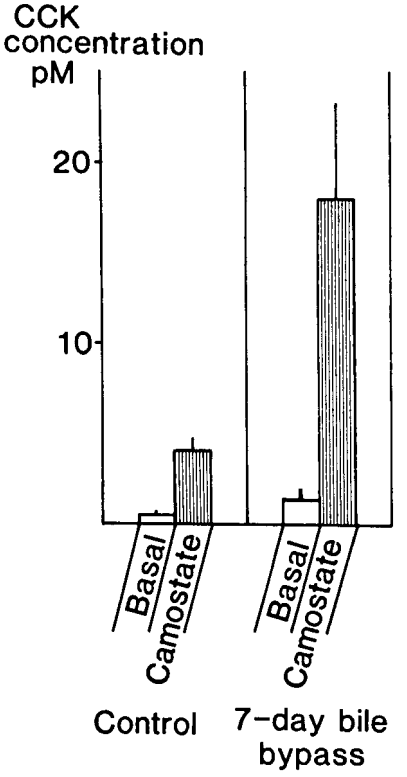

Figure 3. Changes in plasma CCK concentrations before and after camostate ingestion in control and bile-diverted rats. There were significant effects by both treatments of bile diversion and camostate administration by two-way analysis of variance (F values were 26.3 and 21.1, respectively, $P < 0.01$). An interaction between these treatments was significant ($F = 21.1$, $P < 0.01$). $n = 5$–7 for each group.

Administration of camostate induced about a 10-fold increase in plasma CCK concentration in both control and bile-diverted rats. These increases were statistically significant (Fig. 3). The integrated response of CCK release was significantly higher in rats with bile diversion than in control rats (4 vs 17 pmole). The plasma CCK concentration produced by administration of camostate was significantly higher in bile-diverted rats than in control rats (Fig. 3). The value produced by simultaneous treatment of bile diversion and camostate administration was higher than the sum of respective values produced by bile diversion and by camostate. Thus, an interaction between bile diversion and camostate administration was significant.

Histologic Changes. There was no significant difference in the histologic findings of the pancreas between the two groups.

Discussion

Our results showed that the removal of bile from the proximal intestine increased duodenal content of CCK and plasma CCK concentrations. The gel filtration patterns of duodenal extract were different between control rats and bile diversion rats. We observed that the first peak eluted earlier than CCK-33, which is considered to be CCK-58 in both control and bile-diverted rats. CCK-58 has been reported to be the major form of biologically active CCK found in the human, pig, canine, and rat intestines (22–25). Because the increase in the larger molecular forms of CCK in the duodenum by bile diversion was significant, whereas that in the smaller ones was not (Fig. 2 and Table 2), we suggest that the synthesis of CCK in the duodenum was enhanced. The mechanism to increase duodenal CCK content remains unknown, but it is suggested that the release of CCK was stimulated by the exclusion of bile, so that the synthesis of CCK was accelerated to maintain the high level of peptide release.

We have reported that pancreatic hypersecretion occurred when trypsin activities in the lumen decreased to a level lower than 10% of normal level (20). It is also known that the hypersecretory response of the pancreas to the decrease of luminal trypsin is mediated by CCK (2, 5, 6). Therefore, a mere decrease of trypsin activity could not explain the increase in basal plasma CCK concentration during bile diversion, because trypsin

activities in the lumen in rats with bile diversion were maintained at the level nearly one-half that of control rats. Thus, we would propose that the bile has another regulatory action on CCK release, in addition to stabilizing luminal protease activities, as previously suspected (3). We have recently observed that the high concentration of luminal bile acid (taurocholate) inhibited the effect of luminal stimulants, such as monitor peptide (pancreatic secretory trypsin inhibitor-61) (26). This peptide can release CCK by a mechanism independent of the inhibition of luminal trypsin activity (26, 27). In contrast to this phenomenon, an absence of bile might enhance the luminal stimulation for CCK release by monitor peptides and/or CCK-releasing peptide (28, 29).

The intragastric administration of camostate released a much greater amount of CCK in bile-diverted rats than in control rats. This may be due to the increase in duodenal CCK content and to the decrease in luminal trypsin activity produced by bile diversion, because the smaller the content of pancreatic protease in the intestine is, the more complete the reversal of feedback inhibition will become (30). The evidence that the decrease of luminal bile acid or bile enhanced the stimulatory effect of luminal administration of trypsin inhibitor is compatible with the report by Brand and Morgan (31). They observed that the response of pancreatic growth to the feeding cholestyramine and soybean trypsin inhibitor together was greater than that seen after feeding either trypsin inhibitor or cholestyramine alone. Moreover, they reported (31) the interaction of dietary trypsin inhibitor and cholestyramine, an anion exchange resin that binds bile salts. They reported that chronic feeding of cholestyramine or chronic bile diversion (4 weeks) resulted in a significant increases in pancreatic weight, DNA, and protein content. However, in our study, these factors were not changed, although pancreatic content of chymotrypsin significantly increased and amylase content significantly decreased. These differences may be attributed to the shorter treatment period in our study.

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