Chronic Bile Diversion to the Urinary Bladder Enhances Cholecystokinin Release and Suppresses Gastric Inhibitory Polypeptide Release in Dogs

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TAKAHASHI, M., SASAKI, I., NAITO, H., FUNAYAMA, Y., SHIBATA, C. and MATSUNO, S. *Chronic Bile Diversion to the Urinary Bladder Enhances Cholecystokinin Release and Suppresses Gastric Inhibitory Polypeptide Release in Dogs.* Tohoku J. Exp. Med., 1996, 178 (2), 151-162 — The role of intestinal luminal bile on cholecystokinin (CCK) and gastric inhibitory polypeptide (GIP) release was examined in dogs with chronic external bile diversion. In 6 mongrel dogs, cholecysto-jejuno-cystostomy (C-J-C) was performed using a small segment of the middle small intestine interposed between the gallbladder and urinary bladder with section of the common bile duct. Butter dissolved in 30 ml of lukewarm water was orally ingested before surgery, and same amount of butter solution with or without graded volumes of canine bile were orally ingested between four to six weeks after C-J-C surgery. Increases in both plasma triglyceride and GIP levels after butter ingestion were almost completely abolished by C-J-C, but they were restored by oral bile feeding in a volume-dependent manner. Both basal and fat-stimulated CCK release were enhanced significantly by C-J-C, and oral bile feeding inhibited fat-stimulated CCK release by bile in a dose dependent manner. These data suggest that intraluminal bile regulates basal and fat-stimulated CCK release and fat-stimulated GIP release in dogs. ——— biliary diversion; cholecystokinin; gastric inhibitory polypeptide; plasma triglyceride; bile feeding

Intraluminal bile has been hypothesized to control the secretion of several gastrointestinal (GI) hormones including cholecystokinin (CCK) (Gomez et al. 1986), gastric inhibitory polypeptide (GIP) (Imamura et al. 1982, 1988), neurotensin, secretin and others (Green and Nasset 1980; Hanssen 1980; Osnes 1981). However, the precise role of an intact enterohepatic circulation in modulating GI peptides secretion is far from clear.

Results of several studies suggest that luminal bile may play an important role in the secretion of CCK and GIP. Both temporary bile diversion in dogs (Gomez et al. 1986) and oral cholestyramine administration in humans (Koop et al. 1988) enhanced fat-stimulated CCK release, and common bile duct ligation

enhanced basal CCK secretion in rats (Baba et al. 1986). Studies in humans have shown that fat-stimulated GIP release is reduced in patients with cystic fibrosis (Ross and Shaffer 1981) and obstructive jaundice (Imamura et al. 1988).

The above reports have shown the effects of either temporary biliary diversion or pathologic conditions on release of CCK and GIP. Whether effects on CCK and GIP release can be induced by chronic biliary diversion or by liver and pancreatic dysfunction is unknown.

The present study was designed to examine the effect of 1) chronic, total bile diversion to the urinary bladder on CCK and GIP release in conscious dogs without liver or pancreatic dysfunction, and 2) the effect of graded amounts of orally-administered bile on release of these peptides in the face of chronic bile diversion.

MATERIALS AND METHODS

Animal preparation

Six adult mongrel dogs (four male and two female) weighing 14 to 22 kg were used. They were anesthetized using intravenous sodium pentobarbital, and cholecysto-jejuno-cystostomy (C-J-C) was performed.

A segment (about 20 cm long) was taken from mid small intestine and interposed between the gallbladder and urinary bladder, with ligation and section of the common bile duct (Fig. 1). After the operation, the dogs were fasted and received 1,000 ml of Hartman solution with antibiotics intravenously for three days. Thereafter, they were fed carbohydrate rich dog food once a day. Animals were allowed to recover from surgery for 4 weeks prior to experiments.

Three weeks after C-J-C, the average (mean $\pm$ s.e.) of serum total bilirubin

Fig. 1. Cholecysto-jejuno-cystostomy (C-J-C) was performed using a small segment about 20 cm long of the middle small intestine interposed between the gallbladder and the urinary bladder with section of the common bile duct for chronic total biliary diversion.

levels in these 6 dogs was 0.35 ± 0.15 mg/100 ml, GOT level was 23.7 ± 2.5 KU and GPT level was 36.1 ± 5.6 KU. These values were not significantly different from preoperative levels (paired Student's *t*-test). The dogs remained in good condition with good appetite in spite of body weight loss of less than 7% during experiment.

Butter loading test

Prior to C-J-C surgery, dogs were deprived of food for 18 hr. They were then allowed to ingest butter (3 g/kg body weight), dissolved in 30 ml lukewarm water. Dogs consumed the butter in one minute. Blood samples were collected from an antecubital vein before and after butter ingestion (0, 15, 30, 60, 90, 120, 150, 180, 210 and 240 min).

After recovery from the C-J-C surgery, butter loading test was repeated. In separate studies, butter loading tests were conducted in which the lukewarm water was replaced by graded volumes of gallbladder bile (5 ml, 10 ml and 20 ml) obtained from donor dogs. Butter (3 g/kg)-bile solutions were made up to 30 ml with water. Butter loading tests were carried out at least twice a week between 4 and 6 weeks after C-J-C surgery.

Blood samples were collected into chilled tubes containing aprotinin (Trasylol[®], 500 KIU, Bayer, Leverkusen, Germany) and disodium ethylenediaminetetraacetic acid (EDTA) (1.25 mg/ml blood, Wako Jyunyaku Kogyo Co., Ltd., Osaka), then immediately centrifuged (3,000 rpm/10 min). Plasma was stored at -20°C .

Before and 3 weeks after C-J-C surgery, feces were collected immediately after defecation for assay of fecal chymotrypsin activity. Feces were stored at -20°C .

Determinations

Plasma triglycerides were measured enzymatically (Determiner TG[®] assay kit; Kyowa Medix Inc., Tokyo) using an auto analyzing system VP super (Dinabot Co., Ltd., Tokyo).

Plasma CCK concentration was measured by means of a radio-immunoassay using CCK-8 N-terminal specific antibody and OAL-656 (Himeno et al. 1983) from Otsuka Assay Laboratories (Tokushima). The antiserum specifically reacted with an N-terminal region of CCK-8, and bound 100% of both CCK-8 and CCK-33, and 85% of CCK-39. It did not cross-react with non-sulfated CCK-8, either sulfated or non-sulfated form of gastrin-17, or other gastrointestinal peptides (Fig. 2). Synthetic CCK-39 coupled to ^{125}I hydroxyphenyl propionic acid-succinimide ester (Bolton-Hunter reagent, Otsuka Seiyaku Co., Ltd., Tokushima) was used as tracer, and synthetic pure porcine sulfated CCK-33 purchased from Peptide Institute Inc. (Osaka) was used as standard.

Plasma samples were extracted with 96% ethanol (1 : 2 vol/vol) (Iwai et al.

Fig. 2. Displacement curves of CCK-related peptides and other gut hormones with antibody OAL-656 (CCK-8, sulfated form; Glucagon, pancreatic glucagon)
 ○ CCK 33; ● CCK 8; △ gastrin 17; ▲ glucagon; × GIP.

1986). The CCK assay was carried out in 0.03 M phosphate buffer, pH 7.6, containing 20 mM EDTA, 200 KIU/ml aprotinin, 0.1% Bovine Serum Albumine (BSA) and 0.02% NaN₃. For the assay, 0.1 ml of antibody plus 0.1 ml of standard or unknown samples were preincubated for 18 hr at 4°C with 0.1 ml of 1% normal rabbit serum. Then, 0.1 ml of ¹²⁵I-CCK-39 was added and the mixture was incubated for 48 hr. Second antibody, 0.1 ml of anti-rabbit goat IgG (Seikagaku Kogyo Co., Ltd., Tokyo), was added and the mixture was incubated for 3 hr. Finally, 0.5 ml of 8% polyethylene glycol (PEG4000) was added and the mixture was incubated for 24 hr at 4°C. After this final incubation, the reaction tubes were centrifuged for 30 min at 3,000 rpm and the radioactivities of precipitates containing the antibody-bound CCK were counted by automatic counter. The half-maximal inhibitory dose (ID₅₀) value for CCK-33 was 80 pmol. The recoveries of CCK-33 added to hormone free plasma before the extraction procedure were 97% and 89% at 25 pmol/liter and 100 pmol/liter, respectively. Intraassay coefficients of variation were 6.4% and 8.8% at 25 pmol/liter and 50 pmol/liter, respectively. Interassay coefficients of variation were 14.8% and 18.2% at 25 pmol and 100 pmol, respectively. All samples were analyzed in duplicate in the same assay. The detection limit was 3.8 pmol/liter plasma.

Plasma levels of GIP were measured in non-extracted plasma using antiserum-GIP (R65) according to Moody's method (Lauritsen et al. 1980).

Fecal chymotrypsin levels were determined enzymatically with Monotest Chymotrypsin® (Boehringer Mannheim Corp., Mannheim, Germany).

Integrated responses of gut hormones after butter ingestion were calculated as the area between the basal value and the plotted curve.

Statistical analysis

The observed values were expressed as mean $\pm$ s.e., and statistical analysis for significance was performed by paired *t*-test for liver function, basal plasma levels of CCK and GIP and fecal chymotrypsin levels. One-way analysis of variance (ANOVA) followed by Fisher's comparison test was accepted in comparison with integrated responses of CCK and GIP. Differences were considered significant if $p < 0.05$.

RESULTS

Plasma triglyceride

Before C-J-C surgery, plasma level of triglyceride (TG) increased gradually from fasting levels of 32.1 ± 4.4 mg/100 ml to a peak of 65.3 ± 15.4 mg/100 ml, by 150 min after butter ingestion. After C-J-C surgery, TG levels did not increase after butter ingestion, but with bile feeding TG levels increased in a volume-dependent manner. Plasma TG response to butter feeding with 10 ml of bile was restored to the preoperative state. After 20 ml of bile, TG values were higher than preoperative levels, reaching a peak value of 97.0 ± 19.0 mg/100 ml at 180 min (Fig. 3).

Plasma CCK

Preoperatively, plasma CCK levels showed a gradual increase from a fasting value of 10.1 ± 1.4 pmol/liter to a peak of 24.6 ± 5.6 pmol/liter, 180 min after butter ingestion. After C-J-C, plasma CCK values in the fasting state

Fig. 3. Changes in mean plasma triglyceride levels after butter ingestions. Six dogs received 3 g/kg butter dissolved in 30 ml of lukewarm water. For bile feeding, water was replaced by graded volumes of canine bile (bile volume was 5 ml, 10 ml, or 20 ml) after C-J-C. Values are expressed as mean $\pm$ s.e.
 ○ control; ● C-J-C; △ C-J-C, Bile 5; ▲ C-J-C, Bile 10; × C-J-C, Bile 20.

significantly increased to over 20 pmol/liter (average value was 24.7 ± 1.8 pmol, $p < 0.01$), and rapidly reached a peak of 51.7 ± 9.1 pmol/liter at 120 min after butter ingestion without bile feeding. While bile feeding tended to suppress CCK release by the addition of bile in a volume dependent manner, the plasma CCK values with bile feeding increased similarly by 30 min after butter ingestion but then decreased depending on bile volume. Twenty ml of bile feeding reduced the plasma CCK value to 31.1 ± 8.0 pmol/liter at 120 min after butter ingestion (Fig. 4).

Fig. 4. Changes in plasma cholecystikinin levels after butter ingestion. Six dogs received 3 g/kg butter dissolved in 30 ml of lukewarm water. For bile feeding, water was replaced by graded volumes of canine bile (bile volume was 5 ml, 10 ml, or 20 ml) after C-J-C. Values are expressed as mean $\pm$ s.e.
 ○ control; ● C-J-C; △ C-J-C, Bile 5; ▲ C-J-C, Bile 10; × C-J-C, Bile 20.

Fig. 5. Integrated cholecystikinin responses (mean $\pm$ s.e.) to 3 g/kg butter dissolved in 30 ml of lukewarm water. Water was replaced by graded volumes of bile after C-J-C surgery. Asterisks indicate significant differences. (* $p < 0.05$)

Fig. 6. Changes in plasma gastric inhibitory polypeptide levels after butter ingestion. Six dogs received 3 g/kg butter dissolved in 30 ml of lukewarm water. For bile feeding, water was replaced by graded volumes of bile after C-J-C surgery. Values are expressed as mean $\pm$ s.e.
 ○ control; ● C-J-C; △ C-J-C, Bile 5; ▲ C-J-C, Bile 10; × C-J-C, Bile 20.

Fig. 7. Integrated gastric inhibitory polypeptide responses (mean $\pm$ s.e.) to 3 g/kg butter dissolved in 30 ml of lukewarm water. Water was replaced by graded volumes of bile after C-J-C surgery. Asterisks indicate significant differences. (* $p < 0.05$; ** $p < 0.01$)

The area under the plasma CCK response curve represents the cumulative release of peptide over the course of the experimental period (Fig. 5). Prior to C-J-C surgery, this integrated response to butter feeding was 2.4 ± 0.7 nmol $\cdot$ 240 min. After surgery the cumulative response increased more than 2-folds. Bile feeding reversed this effect in a volume-dependent manner.

Plasma GIP

Fasting values of plasma GIP were similar in all groups, approximately 100 pg/ml (Fig. 6). Before C-J-C surgery, plasma GIP markedly increased after butter ingestion, reaching $1,833 \pm 309$ pg/ml at 90 min. This rise was sustained for the entire study period. After C-J-C surgery, the increase in plasma GIP after butter ingestion was completely abolished. The plasma GIP response to butter ingestion was restored by bile feeding in a volume dependent manner. The highest dose of bile (20 ml) restored the plasma GIP pattern to that of the preoperative state.

The integrated response of GIP over the experimental period (Fig. 7) reflected the release pattern described above: the increase observed in response to butter ingestion prior to surgery was abolished by C-J-C surgery, but was restored by increasing volumes of bile.

Fecal chymotrypsin activity

Before C-J-C surgery, average fecal chymotrypsin activity was 27.9 ± 5.1 U/g. Three weeks after C-J-C surgery, chymotrypsin activity decreased significantly to 2.6 ± 0.45 U/g.

DISCUSSION

Available evidence indicates that pancreatic juice is required for digestion of triglycerides (TG), while luminal bile is necessary for efficient uptake of products of triglyceride hydrolysis by expanding oil-water interfacial area of emulsion droplets. Some reports indicated that luminal hydrolysis of TG was impaired in bile-deficient rats (Cesano and Dawson 1966), but others said unimpaired in bile fistula rats (Morgan and Borgström 1969), bile-deficient dogs (Knoebel and Ryan 1963) and bile fistula humans (Porter et al. 1971). Low dose of fatty acids was absorbed in the absence of bile in humans (Porter et al. 1971), and absorbed fatty acids were transported predominantly via the portal vein (Blomstrand et al. 1969). Therefore, some extent of free fatty acids (FFA), not monoglyceride, can be absorbed in bile fistula dogs. In the present study, C-J-C respectably abolished plasma TG increase after ingestion of butter, and bile feeding restored TG increase in a bile-volume-dependent manner. In C-J-C, luminal hydrolysis of fat by pancreatic juice was considered to be unimpaired as well as dogs or humans with bile fistulas. Therefore, we can reason that bile feeding with butter ingestion increased plasma TG levels in a volume-dependent manner by expanding oil-

water interfacial area of emulsion droplets.

Administration of protease inhibitor (Göke et al. 1986), bile duct ligation (Baba et al. 1986) or bile-pancreatic juice diversion (Nakamura et al. 1990) cause augmentation of basal CCK levels in rats. In addition, dogs with temporary bile diversion (Gomez et al. 1986) or humans with obstructive jaundice (Koide et al. 1993) increase fat stimulated CCK release. Feedback regulation of CCK release by intraluminal protease activity, mainly trypsin, has been established in rats (Miyasaka and Kitani 1986). Decreased intraluminal protease activity by pancreatic juice diversion or oral protease inhibitor administration in rats is considered to enhance CCK release. But it is still unclear why CCK hypersecretion occurs in dogs with temporary bile diversion and in humans with obstructive jaundice. In order to understand the mechanism in these conditions, we should consider the relationship between CCK and luminal trypsin activity. Owyang et al. (1986) reported that intraduodenal trypsin infusion reduced phenylalanine induced CCK secretion in man. However, Hotz et al. (1983) and Sale et al. (1977) reported that oral administration of camostat, a protease inhibitor, did not enhance CCK release in dogs and humans. Oral camostat administration is considered to suppress luminal trypsin activity strongly, but not strongly chymotrypsin activity. Luminal chymotrypsin activity was restored in a short time (for one or two hr) after camostat administration in humans (Dlugosz et al. 1983); thereafter camostat probably does not affect plasma CCK release in dogs and humans. Green and Nasset (1980) have reported that bile kept intraluminal protease activities by preventing autodigestion of both trypsin and chymotrypsin, so bile exclusion from the intestine may induce autodigestion of both trypsin and chymotrypsin. Since the average value of fecal chymotrypsin level after C-J-C decreased to one tenth of the value before C-J-C, trypsin activity may also decrease. We can consider that the lowered activities of both trypsin and chymotrypsin may enhance basal and fat stimulated CCK release after C-J-C.

Intraluminal bile may have a direct inhibitory role on CCK release in rats, because inhibition of intraluminal protease activities alone did not stimulate CCK release as much as did biliary diversion in rats (Nakamura et al. 1990). An inhibitory effect of oral bile feeding on fat-stimulated CCK release in our experiment is consistent with previous reports (Gomez et al. 1986, 1988; Koop et al. 1989), and Gomez et al. (1986) also speculated direct inhibitory role of bile involving CCK release. We could not determine actual intraluminal trypsin and chymotrypsin activities, but we should consider the possibility that bile may have a direct inhibitory role on CCK release, which is independent of intraluminal protease activities.

Lypolysis of fat by pancreatic enzyme is indispensable for fat stimulated GIP release. Ross and Shaffer (1981) reported that TG did not stimulate GIP release in children with cystic fibrosis who had impaired exocrine pancreatic function. Supplementation with pancreatic enzymes in these children produced a GIP

response similar to that seen in normal children. Those authors further examined the importance of the products of triglyceride hydrolysis for GIP release in normal volunteers. The integrated total GIP response to long-chain fatty acids was 2.5-fold lower than that of an equimolar dose of TG, and there was no increase after medium-chain fatty acids and glycerol. From these results, Ross and Shaffer (1981) suggested that hydrolytic products of TG, mainly long-chain fatty acids, were GIP secretagogues.

Bile also has an important role on GIP release. C-J-C suppressed butter-stimulated GIP release, while bile feeding in C-J-C restored GIP release in a bile-volume-dependent manner. Patients with external biliary drainage (Imamura et al. 1988) and dogs with cholecysto-ileostomy (Tsuchiya et al. 1987) also suppressed fat-stimulated GIP release, and 20 ml of bile feeding in cholecysto-ileostomy dogs restored butter-stimulated plasma GIP levels to the level as before surgery. Lypolysis of fat was unlikely to be impaired in C-J-C dogs and changes in plasma GIP levels with bile feeding paralleled with that of plasma TG levels. Combining Ross and Shaffer's (1981) results with ours, we conclude that absorption of digested fat in the form of long chain fatty acids into epithelial mucosal cells which requires micelle formation by bile is indispensable for fat-stimulated GIP release in dogs.

The respective responses of CCK and GIP to dietary fat were different. Watanabe et al. (1988) indicated that intraduodenal corn oil administration in pancreatic duct ligated (PDL) dogs resulted in gradual increase in plasma CCK levels, and corn oil predigested with pancreatic enzyme or oleic acid administration in PDL dogs resulted in immediate and large responses as well as in control dogs. Lypolytic products in the form of long-chain fatty acids are considered to be able to release CCK and absorption of fatty acids appears to be unnecessary for CCK release, because C-J-C enhanced butter-stimulated CCK release but suppressed the plasma TG response to butter feeding, and bile feeding resulted in suppression of CCK and elevation of TG. On the other hand, fat-stimulated GIP release is considered to require absorption of lypolytic products in the form of long-chain fatty acids, because C-J-C suppressed fat-stimulated GIP release and bile feeding in C-J-C restored plasma GIP levels associated with plasma TG levels.

Thus, chronic total biliary diversion enhanced basal and fat stimulated CCK release and suppressed fat stimulated GIP release in dogs. Additionally, bile feeding in these dogs enhanced fat-stimulated GIP release and inhibited fat-stimulated CCK release. In conclusion, the present results support the hypothesis that intraluminal bile regulates CCK and GIP release in dogs.

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