Relationship between gallbladder bile concentration and motility in conscious dogs: role of cholecystokinin

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MURAMATSU, S., K. SONOBE, A. MIZUMOTO, T. YAMADA AND Z. ITOH. Relationship between gallbladder bile concentration and motility in conscious dogs: Role of cholecystokinin. PEPTIDES 18(1) 000–000, 1997.—The relationship between gallbladder (GB) bile concentration and motility was studied in conscious dogs. The 12-h GB bile concentrations between meals could be divided into three periods: diluting, minimum, and concentrating periods. During the diluting period, inhibition of GB contractions by a CCKβ receptor antagonist, atropine or hexamethonium, resulted in concentration of GB bile, whereas during the concentrating period, CCK-8 shifted the concentration process back to dilution. The GB appears to absorb water continuously from GB bile, which is not regulated by cholinergic or CCKβ receptors. The postprandial progressive dilution of GB bile is brought about by GB pumping controlled by cholecystokinin (CCK).

Cholecystokinin L-364,718 Atropine Hexamethonium Gallbladder motility Gallbladder bile concentration

THE main function of the gallbladder (GB) is to serve as a reservoir for bile and facilitate evacuation of bile when it is needed for digestion. The reservoir function of the GB is enhanced greatly by its absorptive capacity, which results in a 10-fold decrease of the original volume of hepatic bile delivered to it (7). In a previous study (31), we demonstrated that when dogs are fed regularly, the GB bile concentration decreases quickly in response to feeding and then gradually increases again to the prefeeding level before the next feed, but we were unable to determine what controls these GB bile concentration changes. In recent studies, GB contractile activity has been suggested to play an important role in controlling the concentration of GB bile (1,10,15). With regard to the concentrating activity of the GB, neural and humoral control of fluid transport across the GB epithelium has been studied in various ways (19,36). These studies, most of which were carried out on anesthetized animals or in vitro, have elucidated a number of mechanisms involved in GB bile concentration control. In experiments on anesthetized animals, however, it is impossible to study GB function in response to feeding, which is of great importance to the study of the true physiological mechanisms that control digestive organ function. Although there are many limitations to the methods that can be applied in conscious dogs, we consider it important to know the changes in GB bile concentration between meals in relation to GB contractile activity. Because there have been very few studies describing these changes in conscious animals, we extended our study to clarify the inhibitory effect of GB contractile activity on GB bile concentration in conscious dogs. Cholinergic pathways and cholecystokinin (CCK) are well known as important factors for the control of GB contractions as a neural and a hormonal factor, respectively. In the present study, therefore, we examined the effects of anticholinergic agents and CCK-related compounds on GB bile concentration in relation to changes in GB contractile activity.

METHOD

Preparation of Animals

Five healthy adult mongrel dogs weighing 12–16 kg were used in this study. Under general anesthesia by IV injection of pentobarbital sodium (Nembutal, 30 mg/kg body wt., Abbott Laboratories, Chicago, IL), the abdominal cavity was opened. A silastic tube (100–1N, Kaneka Medix Corp., Osaka, Japan) was inserted into the GB through an incision in the fundus so that the tip lay 10 mm from the GB wall and was secured with a purse-string suture. This tube (called the GB tube) was used to aspirate GB bile. A force transducer (12) was sutured to the serosa of the GB body oriented to measure circular muscle contractions. Two other force transducers were sutured similarly to the serosa of the gastric antrum 3 cm proximal to the pyloric ring and duodenum 3 cm distal to the opening of the pancreatic duct to measure circular muscle contractions. The lead wires of the three

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force transducers and the GB tube were pulled out together through a skin incision on the back and secured to the adjacent skin with silk sutures. After confirming its patency, the GB tube was filled with saline and sealed with a plastic plug. After the abdominal surgery, a silastic tube (602-205, Dow Corning Corp., Midland, MI) was inserted into the superior vena cava through a branch vein of the right external jugular vein, filled with heparinized saline, and sealed with a plastic plug. The external end of the tube in the jugular vein was also secured to the adjacent skin. This tube (called the jugular tube) was used for IV injection and infusion of test materials. After the operation, a jacket protector was placed on each dog to protect the lead wires and the silastic tubes from damage due to the dog scratching. In an additional three dogs, laparotomy was carried out in a similar way and a polyethylene catheter (3 Fr; i.d. 0.8 mm, o.d. 1.0 mm, ATOM Co. Ltd., Tokyo, Japan) was chronically inserted into an appropriate bile duct in the right lobe, and kept in place with silk sutures for continuous collection of hepatic bile to monitor changes in the bilirubin concentration of hepatic bile between meals. After surgery, the tube was exteriorized through the lateral abdominal wall, and attached to a 10-ml rubber bag, which was placed in a small pocket of the jacket protector. The dogs were housed in individual experimental cages, maintained with an IV drip infusion (50 ml/kg body wt.) of Solita T3G (Shimizu Pharmaceutical Co. Ltd., Shizuoka, Japan) for 3 days postoperatively and then gradually returned to their normal diet. During the experimental period, the dogs were fed twice a day at 1000 and 2200 h with a dry pellet type dog food (10.0 g/kg body wt., Gaines meal, Ajinomoto-General Foods Corp., Tokyo, Japan) and allowed free access to water.

**Measurement of Bile Concentrations**

The GB bile concentrations of bile specimens aspirated through the GB tube were measured. First, the GB tube was con-

FIG. 1. Twelve-hour changes in gallbladder (GB) bile bilirubin and sodium concentrations in conscious dogs in response to feeding. Each GB bile concentration value in this and the following figures represents the means ± SEM obtained from two experiments in each of five dogs. The dogs were fed every 12 h with half the volume of dog food necessary for their daily calorie requirements. The 12-h changes could be divided into three periods, according to the bile concentration: diluting, minimum, and concentrating periods.

**Measurement of Contractile Activity**

Gastric, duodenal, and GB contractile activities were recorded simultaneously from 0900 to 2200 h continuously using a multichannel pen-writing recorder (ME-175D, Nihon Kohden Kohgyo Co., Ltd., Tokyo, Japan) after the cable leads from the amplifiers (UG-6, Nihon Kohden Kohgyo Co. Ltd., Tokyo, Japan) had been connected to the lead wires from the force transducers under the protector. The cable leads were suspended from the ceiling just above each cage, which allowed the dogs to move freely in the experimental cages. The recordings of contractile activity in the gastroduodenal regions and GB were used to determine the general contractile activity, but when more detailed information about contractile changes were needed, the signals from the amplifiers were recorded on a tape recorder (RD-110T, TEAC, Co., Tokyo, Japan). The detailed changes in GB contractile activity have been reported previously (31).

**FIG. 2.** Effects of normal saline (A), atropine (B), hexamethonium (C), and the CCKA receptor antagonist L-364,718 (D) on GB and gastroduodenal contractile activity in a conscious dog in response to feeding (indicated by arrows) observed during the diluting period. IV infusion of these inhibitors, started 15 min before feeding, did not influence the dogs’ appetites but the meal-induced GB contractions were virtually abolished by all three agents. Note that, in contrast to its inhibition of GB contractile activity, L-364,718 had no influence on gastroduodenal contractile activity.
FIG. 3. Effects of normal saline (A), atropine (B), hexamethonium (C), and L-364,718 (D) on GB and gastroduodenal contractile activity in a conscious dog during the concentrating period. Atropine and hexamethonium inhibited GB and gastroduodenal contractions, but L-364,718 influenced neither parameter during this period.

FIG. 4. Effects of atropine, hexamethonium, and L-364,718 on meal-induced GB bile concentrations in five conscious dogs. The dilution of GB bile in response to feeding was virtually abolished by pretreatment with each of these inhibitors and the GB concentration continued to increase during and 2 h after their infusions. The concentrating rates of these three inhibitors did not differ significantly.

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\text{GB bile was aspirated at 30-min intervals from 60 min before feeding until the next feed.}
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**Control Experiment**

The 12-h changes in GB bile concentrations were demonstrated in a previous study (31), but were reexamined in this one. This experiment, consisting of contractile activity recording and GB bile aspiration for 13 h, was carried out once or twice a week. During the experiment, the dogs were allowed to move freely as usual in the experimental cages. After connecting the cable leads to the transducers lead wires, GB bile collection was started at

FIG. 5. Effects of atropine and hexamethonium on GB bile concentrations in five conscious dogs during the concentrating period. In comparison with normal saline (control) administration, atropine and hexamethonium did not affect the concentrating activity significantly.
Drugs and Hormonal Preparations

Atropine and hexamethonium were purchased from commercial sources. The CCK$_{A}$ receptor antagonist L-364,718 was a gift from Merck Sharp and Dohme (Rahway, PA). CCK-S was purchased from Peptide Institute Inc. (Osaka, Japan).

Statistics

Means ± SEM are shown. Analysis of variance (ANOVA) followed by Bonferroni correction for multiple comparisons was used for the statistical comparisons.

RESULTS

Twelve-Hour Changes in GB Bile Concentration and Contractile Activity in Conscious Dogs

As shown in Fig. 1, when the dogs were fed regularly twice a day at 1000 and 2200 h with pellet-type dog food to provide

**Effects of Cholinergic Inhibitors and a CCK$_{A}$ Receptor Antagonist**

The inhibitory effects of GB movements on GB bile concentrations during the diluting, minimum, and concentrating periods were examined. To inhibit GB contractions, the muscarinic and nicotinic cholinergic receptor antagonists atropine and hexamethonium, respectively, and the specific CCK$_{A}$ receptor antagonist L-364,718 were used. During the diluting period, these agents were given as bolus IV injections 15 min before feeding followed by an IV infusion for 120 min. GB bile concentrations and contractile activity were measured from 60 min before and for 4 h after feeding. As control for this experiment, normal saline (10 ml/h) was infused IV. During the concentrating period, these agents were given as an IV infusion from 8 to 12 h after feeding. During the minimum period, L-364,718 was given as an IV infusion from 4 to 8 h after feeding.

**Effects of CCK-8 on GB Bile Concentrations**

As the action of CCK on GB movements was found to be likely to dilute GB bile, CCK-8 was given as a 2-h continuous IV infusion during the concentrating period. The dose of CCK-8 that induced physiological GB contractions was selected as described previously (28).
half the necessary daily calories at each feed, the GB bile bilirubin and sodium concentrations fell soon after feeding, reached their minima 3.7 ± 0.5 h (diluting period) after feeding and remained at this level for 4.3 ± 0.5 h (minimum period). At 8.0 ± 0.3 h postprandium, the GB bile concentration started to increase and had returned to the initial preprandial level by the next feeding time (concentrating period duration: 4.0 ± 0.3 h). The GB bile bilirubin concentration during the minimum period was similar to that in the hepatic bile aspirated directly from the hepatic bile duct at different time points. The mean hepatic bile bilirubin concentration was 24.1 ± 0.6 mg/dl in the diluting period, 23.8 ± 0.9 mg/dl in the minimum period, and 24.7 ± 0.8 mg/dl in the concentrating period, and these concentrations were not significantly different from each other. As the GB bile bilirubin and sodium concentrations always changed in parallel, only bilirubin concentrations were measured in the subsequent experiments. In association with these GB bile concentration changes, the GB contraction pattern also changed: there was an immediate rise in tonic activity in response to feeding followed by maintained elevated tonic activity for 8.5 ± 0.6 h after feeding [Fig. 2(A)]. Phasic contractions with a frequency of 4.0 ± 0.1 to 4.5 ± 0.1 contractions/minute were always superimposed on the increased GB tonic contractile activity, as reported previously (31). After this period, the elevated tonic activity gradually decreased and 8.5 ± 0.6 h after feeding, the contractile pattern became irregular, as shown in Fig. 3(A).

Effects of Inhibition of GB Contraction on GB Bile Concentrations During the Diluting Period

The effects of suppressing GB contractions by administering two cholinergic inhibitors and a specific CCK4 receptor antagonist on the GB bile concentrations were examined. Continuous IV infusions of each of these three agents inhibited the postprandial rise in GB tonic activity and phasic contractions strongly [Fig. 2(B–D)] and the GB bile was not diluted, but was concentrated further during and after these IV infusions without the concentrating rates of these three inhibitors differing significantly (Fig. 4). Gastroduodenal contractile activity was suppressed strongly by atropine and hexamethonium [Fig. 2(B, C)]; whereas L-364,718 did not affect the postprandial gastroduodenal contractile activity [Fig. 2(D)].

Effects of Inhibition of GB Contraction on GB Bile Concentrations During the Minimum and Concentrating Periods

When the cholinergic blockers were given during the concentrating period, the concentrating rates were not significantly affected at all, as shown in Fig. 5, but GB contractions were inhibited by these two agents, as shown in Fig. 3(B, C). In contrast, L-364,718 given during the concentrating period induced no changes in the contractile activity of the GB, stomach, or duodenum [Figs. 3(D), 6(A)], nor did it affect the GB bile concentration [Fig. 7(A)]. However, when L-364,718 was given during the minimum period, the GB bile concentration started to increase significantly earlier than after normal saline administration [Fig. 7(B)] and the GB contractile activity, particularly the tonic activity, was inhibited dramatically [Fig. 6(B)]. Furthermore, gastroduodenal contractile activity was increased by L-364,718 [Fig. 6(B)].

Effects of CCK-8 on GB Bile Concentrations During Concentrating Period

As we found that CCK appeared to dilute GB bile by stimulating GB pumping, the effects of CCK-8 (70 ng/kg/h)–induced GB contractions on GB bile concentrations during the three different phases were studied and the results are shown in Figs. 8 and 9. When CCK-8 was given at the end of the minimum period, the GB bile concentration remained at its minimum level and was not concentrated further significantly [Fig. 8(A)]. When CCK-8 was given during the period when the GB bile was fairly concentrated, the GB bile concentration stopped rising and declined [Fig. 8(B)]. When CCK-8 was given at the end of the concentrating period, namely in the fasting state, no further concentration of GB bile was observed and the concentration remained at the level it was when the CCK-8 infusion was initiated [Fig. 8(C)]. The CCK-8–induced GB contractions corresponding to these GB bile concentration changes are shown in Fig.9. The GB contractile response to CCK-8 during the fasting state...
tractions started to decrease within 30 min after feeding, reached their minimum levels rapidly within 2–3 h, and this lasted for several hours thereafter. The GB bile concentration then started to increase and returned to its original concentration by the time of the next feed (31). These GB bile concentration changes were confirmed in the present study, and our aim was to examine what controls the changes in GB bile concentration. The precise measurement of water flux across the GB epithelium should be carried out with the cystic duct closed and, therefore, we did not attempt to measure the absolute transport of water in the GB in this study, but investigated the effects of anticholinergic agents and CCK-related compounds on GB bile concentration in relation to GB motility.

An important point that should be clarified before we proceed to the main issue is the hepatic bile concentration between meals and the mechanism of action of CCK in the GB. Although we determined the hepatic bile concentration during three different periods between meals, the bilirubin concentration in these different periods did not differ significantly, but was rather constant in the region of 24 mg/dl. Therefore, we consider it likely that the changes in GB bile concentration are due to absorption and/or secretion of water from the GB, and not due to inflow of hepatic bile at different concentrations. With regard to the mechanism of action of CCK, as our group (28,32) and others (5,9,22,25,33) have reported, GB contractions in response to CCK in vivo are assumed to be controlled mainly by endogenous acetylcholine released as a result of a long vago-vagal reflex activating CCK receptors on vagal afferents. However, as CCK receptors have been identified in the gallbladder smooth muscle layer per se (26–28), direct stimulation of GB smooth muscle by CCK cannot be ruled out. CCK-induced GB contractions can be abolished by a CCK receptor antagonist and partially inhibited by atropine and hexamethonium at low doses (28). In the present study, therefore, we used doses of these cholinergic blockers that inhibited gastrointestinal contractions.

When L-364,718 was given continuously starting 15 min before feeding, the GB did not contract in response to feeding. The inhibitory effect of L-364,718 on GB contractions lasted for several hours after stopping the IV infusion and, simultaneously, the normal postprandial dilution of GB bile was shifted to concentration. As L-364,718 has not been reported to affect water transport in the GB, the shift from dilution to concentration evoked by L-364,718 has been postulated to be due to inhibition of GB movements. It is, therefore, feasible that the postprandial GB bile dilution was brought about by postprandial GB contractions, which were probably induced by CCK.

When the postprandial GB contractile activity was abolished by atropine or hexamethonium, the effects on GB bile concentration were quite similar to that seen with L-364,718: food-induced GB bile dilution was disrupted and the GB bile was concentrated continuously thereafter. These findings suggest that postprandial GB bile dilution was brought about by postprandial GB contractions and that the absorption of water by the GB was not inhibited by cholinergic or CCK receptor antagonists even after ingestion of a meal. Svanvik et al. (30) attempted to correlate meal-stimulated absorptive function in monkey gallbladders and, in contrast to our data, they concluded that net water secretion occurred after feeding, although we cannot comment on these discrepancies due to the inherent methodological and species differences.

To test our hypothesis that the absorptive activity of the GB is not affected by cholinergic and CCK receptor antagonist, atropine, hexamethonium, and L-364,718 were given during the concentrating period and all three agents were found not to affect the concentrating activity of the GB at all during this period.
These data support the previous findings that acetylcholine and CCK do not influence net water absorption by the GB (4,14). However, if an IV infusion of L-364,718 was initiated 4 h after feeding during the minimum period, that is 2–3 h earlier than the start of the concentrating period, the GB bile concentration started to increase 90 min after starting the infusion, significantly earlier than it did after normal saline administration, indicating that the disappearance of postprandial movements due to the elimination of CCK activity and acceleration of GB refilling (6) enabled the GB to shift from dilution to concentration of bile. These findings support our view that the dilution of GB bile observed mainly during the postprandial period is caused by GB pumping, which is probably regulated by CCK (20,21).

In the present study, the postprandial GB movements were found to affect dilution of GB bile, so we examined the effects of CCK on GB contractile activity and GB bile concentration. A physiological dose of CCK always increased tonic activity of the GB, on which regular phasic contractions were superimposed, and the GB bile concentration decreased progressively as these contractions continued. The best example of this phenomenon was seen when CCK was given during the concentrating period, which resulted in GB bile dilution. However, if CCK was given at the end of the concentrating period, namely in the fasting state, as shown in Fig. 8 (C), the GB bile concentration did not change and remained at the same level as it was when the CCK infusion started. These different effects of CCK on GB bile concentrations during different phases could be ascribed to the different contractile patterns induced by CCK. As shown in Fig. 9 (C), CCK-induced tonic contractions during the fasting state were not accompanied by phasic contractions; the increase in torque certainly emptied the GB to some extent, but the bile concentration did not change unless the GB engaged in pumping. These results are in agreement with those of Abiru et al. (1), who measured bile flow in the cystic duct using a sophisticated technique in which CCK could not induce alternate GB filling and emptying excursions in a fasting state. Therefore, we assume that the small phasic contractions superimposed on the increased tonic GB contractions are definitely involved in GB bile dilution by pumping bile in and out of the GB and consequently, the GB bile concentration declines to that of the hepatic bile as long as the plasma CCK level is elevated. What, however, controls the small phasic contractions superimposed on the tonic GB contractions during the postprandial period? We could not establish the mechanism responsible, but some other factors may be activated by CCK to produce the small phasic contractions observed after meals. The role of CCK in the sphinctor of Oddi should not be neglected in specific postprandial GB contractions.

With regard to the GB bile concentrations, the GB seems to absorb water continuously from the GB epithelium, but when the rate of GB bile dilution due to entry of dilute hepatic bile exceeds the GB concentration rate, as seen during the postprandial period, the GB bile appears to be diluted. Abiru et al. (1) reported that the GB showed alternating excursions of filling and emptying in a postprandial state, and in our study, administration of L-364,718 during the minimum period enabled the GB to shift from dilution to concentration of bile. Therefore, as long as CCK is released, the GB bile is not concentrated, because dilute hepatic bile enters the GB as a result of its pumping function (1,10,15), but as CCK action is reduced, the GB enters the filling phase and the GB bile starts to be concentrated. Similar findings were reported by Traynor et al. (34), who observed that the large amplitude GB pressure elevations prevented GB filling for about 5 h after the first postprandial 2 h.

In the present study, GB contractile activity was suggested to play an important role in controlling the concentration of GB bile. Neural and humoral control of fluid transport across the GB epithelium has been studied in various ways in vivo and in vitro (19,36). Electrical stimulation of the splanchic nerves and adrenoreceptor stimulation has been reported to increase the net water absorption from the gallbladder in anesthetized cats (2,3,17) and in vitro (8,24). The influences of gastrointestinal hormones on GB bile concentrating activity have also been studied: secretin inhibited water absorption in anesthetized cats (14) and in vitro (11,29,35), whereas vasointestinal polypeptide (VIP) induced net water secretion into the lumen (13,16,18,23,24,35). In fact, these neural and humoral controls of GB concentrating activity must theoretically affect GB bile concentration. However, Jazrawi et al. showed that these effects can be negligible, because the changes in GB volume depended on GB contractile activity to a much greater extent than GB concentrating activity (15). Furthermore, acetylcholine and CCK, the most important factors that control GB motility, do not affect GB concentrating activity (4,14). Therefore, it is suggested that the changes in GB bile concentration observed in the present study depend mainly on the changes in GB contractile activity. In conclusion, the postprandial GB bile concentration changes in dogs can be divided into three major periods: diluting, minimum, and concentrating periods. During the diluting period, GB bile is diluted progressively as hepatic bile enters the GB as a result of GB pumping controlled by CCK. When CCK action is reduced, the GB enters the concentrating period.

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