Canine pancreatic juice stimulates the release of secretin and pancreatic secretion in the dog

Y. SONG, P. LI, K. Y. LEE, T.-M. CHANG, AND W. Y. CHEY

The William B. and Shella Konar Center for Digestive and Liver Diseases, University of Rochester Medical Center, Rochester, New York 14642

Song, Y., P. Li, K. Y. Lee, T.-M. Chang, and W. Y. Chey. Canine pancreatic juice stimulates the release of secretin and pancreatic secretion in the dog. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G731–G735, 1999.—A secretin-releasing factor (SRF) was found in canine pancreatic juice that increases plasma secretin and stimulates pancreatic secretion in rats, suggesting that a positive feedback mechanism may be involved in the regulation of pancreatic secretion. In the present study, we investigated to determine whether or not SRF releases endogenous secretin and stimulates exocrine pancreatic secretion in conscious dogs. Fresh pancreatic juice was collected from four dogs by intravenous administration of secretin at 0.5 µg·kg⁻¹·h⁻¹ and CCK at 0.2 µg·kg⁻¹·h⁻¹. The juice was boiled for 10 min at 100°C. Experiments were carried out in phase I of spontaneous cycle of interdigestive pancreatic secretion. The testing solutions were infused intraduodenally in separate experiments: NaHCO₃ solution (0.1 M, 4.5 ml/min, 60 min), a corn oil (Lipomul, 2 ml/min, 10 min), boiled pancreatic juice (BPJ, 4.5 ml/min, 60 min), and mixture of BPJ and Lipomul. Pancreatic secretion of fluid and bicarbonate was significantly increased by either BPJ or a mixture of BPJ and Lipomul (34- and 31-fold or 41- and 38-fold, respectively). Plasma secretin level increased by 164.7 ± 13.4% and 223.1 ± 35.0%, respectively, from basal concentration of 1.7 ± 0.5 pM. In contrast, neither bicarbonate solution nor Lipomul influenced the plasma secretin level or pancreatic secretion. In addition, when Lipomul was incubated with BPJ, no fatty acid was produced. Thus the increased pancreatic secretion in the dog infused with a combination of BPJ and Lipomul was caused by SRF in BPJ, which released endogenous secretin. Moreover, the increases by BPJ of both plasma secretin level and bicarbonate secretion were completely blocked by intravenous administration of an antisecretin antibody in these dogs. The observations suggest that SRF in pancreatic juice exerts a positive feedback effect on exocrine pancreatic secretion that is mediated by the release of secretin in the interdigestive state in dogs.

MATERIALS AND METHODS

Dog preparation. Four mongrel dogs, two males and two females weighing 15–24 kg, were surgically prepared with a Thomas gastric cannula and duodenal cannula as described previously (17). At least 4 wk were allowed for the dogs to recover from surgery, and before each experiment they were fasted for 10 h with free access to drinking water. The gastric cannula was opened during the experiment to allow gastric juice to drain by gravity. A glass tube was inserted into the main pancreatic duct via the Thomas duodenal cannula and was held in place by a rubber stopper at the cannula opening. Canine pancreatic juice preparation. A large quantity of pancreatic juice was collected from the dogs several days before the experiment. Secretin at 0.5 µg·kg⁻¹·h⁻¹ (kindly provided by Dr. David Coy at Tulane University, New Orleans, LA) and CCK-8 at 0.2 µg·kg⁻¹·h⁻¹ (Research Plus, Bayonne, NJ) were infused intravenously to stimulate pancreatic secretion. All of the juice obtained from the dogs (~400–500 ml·dog⁻¹·day⁻¹) was pooled and was immediately boiled for 10 min at 100°C. The boiled pancreatic juice (BPJ) was centrifuged for 20 min at 7,000 rpm to eliminate precipitated protein and mucus. Supernatant was then kept frozen at −20°C until the time of the experiment. Experiments. Animals were placed on Pavlov stands with both cannulas kept open. The upper small intestine was thoroughly washed by infusing warm water at 4.5 ml/min for at least 60 min. Pancreatic juice was collected at 15-min intervals to record the spontaneous interdigestive pancreatic secretory cycle. As soon as the phase III secretory pattern was over, intraduodenal infusion of a testing solution was started in phase I. After a 30-min collection of basal secretion, one of the following testing solutions was infused into the duodenal lumen through one or two plastic tubes (2 mm ID) via a Thomas duodenal cannula: NaHCO₃ solution (0.1 M) at rate of 4.5 ml/min for 60 min; corn oil (Lipomul, Upjohn, Kalamazoo, MI) at a rate of 2.0 ml/min for 10 min; BPJ at a rate of 4.5 ml/min for 60 min; or a combination of Lipomul for 10 min and BPJ for 60 min.

SECRETIN AND CCK are the two major gut hormones that drive pancreatic exocrine secretion. It has been suggested that pancreatic enzyme secretion and the release of CCK in the rat are mediated by both a positive feedback mechanism via a monitor peptide from pancreatic juice (4, 8, 16) and negative feedback via a luminal CCK-releasing factor from small intestinal secretion and a diazepam binding inhibitor in small intestinal extract in the rat (5, 13, 15). A negative feedback mechanism is also operative in pancreatic secretion of fluid and bicarbonate via endogenous release of secretin in dogs as well as rats (7, 10, 11, 18). A secretin-releasing peptide (SRP) has been found and partially purified from rat upper small intestinal extracts (11). Like a monitor peptide (4, 8, 16), a secretin-releasing factor (SRF) was recently found in canine pancreatic juice that released secretin and increased pancreatic secretion in anesthetized rats (12). The finding suggested that a positive feedback mechanism may be operative in regulation of canine pancreatic secretion. The present study was undertaken to investigate a possible presence of SRF in the pancreatic juice that stimulates pancreatic secretion of fluid and bicarbonate in the dog.
To measure plasma immunoreactive secretin, venous blood samples were drawn at 15-min intervals from a peripheral vein in a hind leg via an intravenous infusion catheter that was kept continuously open by slow infusion of 0.15 M NaCl solution. To investigate the effect of an antisecretin antibody on pancreatic secretion with BPJ and/or Lipomul infusion, a rabbit antisecretin serum, with a titer of 1:106, was given intravenously 1 day before the experiment (1.2 ml/dog). Then the experiments with intraduodenal infusion of BPJ or a combination of BPJ and Lipomul were repeated as described. The amount of the antisecretin serum injected was based on our previous studies in the dog (3, 9, 19).

**In vitro study.**

To test lipase activity in BPJ, 2 ml of Lipomul were mixed with 0.5 ml of either fresh pancreatic juice (FPJ) or BPJ and incubated in water bath at 37°C for 30 and 60 min. The enzyme activity was blocked by cooling the testing solution on ice. Free fatty acid derived from hydrolysis of Lipomul was measured as follows: 1 ml of Lipomul mixture was thoroughly mixed with 40 µl of 6 N HCl, 2 ml of 2-propanol, and 8 ml of petroleum ether. After centrifugation for 5 min at 3,000 rpm, the upper organic layer was removed. The pellet was blow dried and reconstituted in 2 ml of ethanol. Each 0.2 ml of aliquot was titrated to an end point pH of 7.0 with 0.01 N NaOH. Serving as standard, oleic acid (Fisher Scientific, King of Prussia, PA) in 0, 10, 20, and 40 µl was added to H2O to become 1 ml. Results are expressed as a mean of each duplicate and expressed as µeq/ml.

**Determinations.** Blood samples were centrifuged at 3,000 rpm for 20 min at 4°C. Plasma was separated in 1-ml aliquots, mixed with protease inhibitors containing 2 mg/ml of soybean trypsin inhibitor, 30 µg/ml of bovine pancreatic trypsin inhibitor (Sigma, St. Louis, MO), and 1.1*10^-8 M D-Phe-L-Phe-L-Arg chloromethyl ketone (Calbiochem, LaJolla, CA), and kept at -20°C until the radioimmunoassay of secretin was performed as described previously (1). The volume of pancreatic juice was measured, and its bicarbonate concentration was determined by a Cl/CO2 analyzer (Beckman Instruments, Fullerton, CA).

**Data analysis.** The data are expressed graphically as means ± SE. For pancreatic flow volume and bicarbonate secretion, the statistical difference between two values at corresponding time points under different experimental conditions was analyzed by employing one-way ANOVA. For integrated pancreatic secretion and plasma secretin level (30 min before and after starting infusion), a statistical significance between the repeated measurements was determined by two-way ANOVA followed by Tukey's test. P < 0.05 was considered statistically significant.

**RESULTS**

Pancreatic secretory response to Lipomul and BPJ. Pancreatic secretions of fluid and bicarbonate in phase I were 0.15 ± 0.05 ml/15 min and 2.53 ± 0.51 µeq/15 min.
respectively. In the control experiment, in which bicarbonate solution was infused intraduodenally for 60 min, pancreatic secretion remained unchanged. Duodenal infusion of Lipomul alone also did not influence pancreatic secretion of fluid or bicarbonate secretion. However, when BPJ was infused into the duodenum or a combination of BPJ and Lipomul was infused, pancreatic secretion increased markedly and peaked at 45 min after the infusion of BPJ or a combination of BPJ and Lipomul began (a 34-fold or 31-fold increase in fluid volume, respectively, and a 41-fold or 38-fold increase in bicarbonate secretion, respectively) (Fig. 1 and Fig. 2).

Plasma secretin concentration in response to Lipomul and BPJ. Basal plasma secretin concentration, 1.7 ± 0.5 pM, was not influenced by either bicarbonate solution or Lipomul alone, whereas BPJ or a combination of BPJ and Lipomul significantly elevated the secretin level starting 15 min after the infusion was initiated, and it peaked at 30 min. As shown in Fig. 3, plasma secretin concentration at 30 min in response to BPJ or a combination of BPJ and Lipomul had increased significantly, by 164.7 ± 13.3% (P < 0.01) and 223.1 ± 35.9% (P < 0.01), respectively.

Effect of antisecretin antibody on pancreatic secretory response to BPJ and/or Lipomul. All four dogs received 1.2 ml of antisecretin serum intravenously 1 day before the experiment to immunoneutralize circulating secretin. The pancreatic secretion in response to BPJ or a combination of BPJ and Lipomul was almost completely abolished (Fig. 4).

In vitro study. Lipomul was incubated with FPJ or BPJ under the same experimental conditions. Free fatty acids produced after incubation with Lipomul alone were insignificant. Incubation of Lipomul with FPJ for 30 min resulted in almost a complete hydrolysis of Lipomul (Fig. 5), whereas the incubation of Lipomul with BPJ produced no significant amount of fatty acids. These findings indicated that the increase in pancreatic secretion by a combination of BPJ and Lipomul was not attributable to digested products of Lipomul in the duodenum.

DISCUSSION

The present study has clearly shown that BPJ in the duodenum stimulated pancreatic secretion of fluid and bicarbonate and increased plasma concentration of secretin. To our knowledge, this is the first report suggesting that a factor or factors in canine pancreatic juice stimulates release of secretin.
juice, but not digestive enzymes, are capable of stimulating the release of secretin and pancreatic secretion of fluid and bicarbonate in the dog. The same BPJ was also found to stimulate pancreatic secretion in the rat (12). Although it has been shown that, for stimulation of pancreatic exocrine secretion, Lipomul, a corn oil, has to be hydrolyzed by pancreatic enzymes into free fatty acids to elevate plasma levels of secretin (19) and CCK (20), in the present study, intraduodenal infusion of either Lipomul incubated with BPJ, which should contain an enzymatic activity, or even BPJ alone equally increased plasma secretin level as well as pancreatic secretion of fluid and bicarbonate. In contrast, Lipomul alone without FPJ in the duodenum failed to influence plasma secretin level or the pancreatic secretion because pancreatic juice was diverted from the upper small intestine, confirming our previous work in the dog (19). These observations strongly suggested that a stimulating factor or factors exist in the pancreatic juice that stimulate the release of secretin from the upper small intestine and thus increase pancreatic secretion of fluid and bicarbonate. Furthermore, because the antisecretin antisera has almost completely abolished the stimulated pancreatic secretion, the pancreatic juice contains a specific SRF factor. Magee and Naruse (14) reported that only FPJ and autodigested juice, but not BPJ, could significantly stimulate pancreatic secretion rich in protein and that trypsin inhibitor abolished the stimulatory effect of the pancreatic juice. They concluded that a part of trypsin molecule in pancreatic juice is the major factor in stimulating pancreatic secretion of protein. In contrast, Hong et al. (6) observed a depression of pancreatic secretion by FPJ in both fasting state and stimulated state with intravenous secretin in a pharmacological dose in the dog, and, in an earlier study, Zucker et al. (21) found no effect of pancreatic juice on pancreatic secretion. The discrepancies between their observations and ours are difficult to explain but may be attributable to the differences between the experiments designed by these workers and the present study.

There have been extensive studies in recent years on the feedback regulation of pancreatic exocrine secretion. The releases of both secretin and CCK are recognized as the main factors in the regulatory mechanisms of pancreatic exocrine secretion (4, 5, 7, 8, 10, 11, 13, 15, 16, 18). In the rat, two different releasing factors for CCK have been found and were purified, one of which originated from rat proximal small intestine and another from rat pancreatic juice (5, 8, 15). A SRF was also found in rat upper small intestinal secretion, which exerts a negative feedback regulation of pancreatic secretion of fluid and bicarbonate mediated by the release of secretin (10). Unlike in the rat and pig, no negative feedback regulation on basal pancreatic secretion has been observed in the dog, but, in the postprandial state, this feedback mechanism was shown to be operative in the dog, mediated by the release of secretin (7). The presence of a SRF in canine pancreatic juice suggested that pancreatic exocrine secretion might also be controlled by a possible feedback regulatory mechanism. It has been recently shown that one of the releasing factors in canine pancreatic juice is pancreatic phospholipase A2 (PLA2), which releases secretin from both secretin-enriched rat duodenal mucosal cell preparation and STC-1 cells (2), a murine intestinal endocrine tumor cell line. The physiological role of pancreatic PLA2 in duodenal lumen on the feedback mechanism is yet to be investigated and defined.

The present study suggests strongly that the pancreatic juice in the duodenum may release secretin and stimulate pancreatic secretion of fluid and bicarbonate in the interdigestive state in the dog, whereas, in the digestive state, it participates in a negative feedback regulation of secretin and pancreatic secretion mediated by the release of secretin (7). This effect of pancreatic juice in the duodenum in the interdigestive state is opposite to that in the rat (10). This difference appears to be attributable to species difference. In addition, the relationship between pancreatic and luminal SRF will have to be investigated in the coming years. At present, however, the relationship between the two SRP remains unknown. The fine balance between the two separate feedback mechanisms may regulate pancreatic exocrine secretion of fluid and bicarbonate in both the interdigestive and digestive states.

We thank Norene Buehner, Joseph Morgan, and Laura Braggin for technical assistance and Patricia Faiello for manuscript preparation.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-25962.

Address for reprint requests and other correspondence: W. Y. Chey, Univ. of Rochester Medical Center, GI Unit, Box 646, 601 Elmwood Ave., Rochester, New York 14642

Received 19 January 1999; accepted in final form 2 June 1999.

REFERENCES


