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

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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 also significantly 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 a rate of 4.5 ml/min for 60 min; corn oil (Lipomul, Upjohn, Kalama-zoo, 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.

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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.

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
Pancreatic juice stimulates release of secretin. The presence of a SRF factor in canine pancreatic juice was confirmed. The release of secretin is mediated by the feedback mechanism in the dog, which was also found in the rat and pig. However, no SRF factor was found in the rat upper small intestine. The discrepancies between the experiments may be attributable to species differences. The relationship between pancreatic and luminal SRF will have to be investigated in the coming years. The fine balance between the two feedback mechanisms may regulate pancreatic exocrine secretion of fluid and bicarbonate in both the interdigestive and digestive states.

REFERENCES


