Interactions between Bile and Pancreatic Juice Diversions on Cholecystokinin Release and Pancreas in Conscious Rats (42976)

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Abstract. **Pancreatic exocrine secretion in the conscious rat is regulated by proteases secreted by the pancreas, and cholecystokinin (CCK) is known to be involved in its mechanism. It has also been reported that the absence of either pancreatic juice or bile in the duodenum could stimulate pancreatic secretion. Therefore, differences in CCK release responding to the exclusion of bile, pancreatic juice (PJ), or both bile and pancreatic juice (BPJ) from the intestine were examined by using a bioassay for cholecystokinin. Plasma CCK levels were increased by all three treatments compared with the basal value, the order of their effects being BPJ>PJ>bile diversion, and CCK concentrations produced by BPJ diversion were much greater than can be explained as simply summed effect of exclusions of bile and PJ. Pancreatic exocrine secretions were significantly increased by PJ and BPJ diversions, but the effect of bile diversion on the pancreas was not statistically significant. An additional infusion of CR-1409 (0.1 mg/rat), one of CCK receptor antagonists, inhibited exocrine function stimulated by BPJ diversion. We conclude (i) BPJ diversion is the strongest endogenous stimulant on CCK release; (ii) the potentiation between bile and PJ diversions is induced on CCK release; (iii) pancreatic protein secretion during BPJ diversion is mainly modulated by CCK.**

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Pancreatic exocrine secretion in the conscious rat is regulated by intestinal proteases secreted by the pancreas (negative feedback control) (1–4). Exclusion of bile and pancreatic juice (BPJ) or pancreatic juice (PJ) increases pancreatic protein secretion (1, 5). Cholecystokinin (CCK) is known to be one of major hormones to mediate the feedback regulation of pancreatic enzyme secretion in rats (6–9).

In the meanwhile, bile has also been reported to be involved in this regulatory mechanism (10, 11). The purpose of this study was to elucidate the differences in CCK release and pancreatic secretion responding to the exclusion of bile and pancreatic juice, separately or together, from the intestine.

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Materials and Methods

Male Wistar rats (308–330 g) and female Sprague-Dawley rats (150–170 g) were obtained from Shizuoka Jikken Dobutsu, Shizuoka, Japan. Soybean trypsin inhibitor (type I-S, chromatographically purified), collagenase (type IV), and hyaluronidase (type I-S) were purchased from Sigma Chemical Co., St. Louis, MO: minimal Eagle's medium amino acid supplement from GIBCO Laboratories, Grand Island, NY; bovine serum albumin, fraction V, from Pentex Products, Kankakee, IL; blue starch polymer (Neo-Amylase Test) from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan; Tris (hydroxymethyl) aminomethane from Wako Pure Chemical Inc., Ltd., Tokyo, Japan; synthetic cholecystokinin-octapeptide sulfate (CCK-8) from Peptide Institute Inc., Osaka, Japan; and octadecylsilylsilica (SEP-PAK C-18) cartridges from Waters Associates, Milford, MA. CR-1409 (sodium salt), DL-4-(3,4-dichloro-benzoyl-amino)-5-(di-*n*-pentyl-amino)-5-oxopentanoic acid, one of CCK receptor antagonists, was a generous gift from Professor L. A. Rovati and Profes-

sor I. Setniker of the Rotta Research Laboratorium, Milano, Italy.

Animal Preparation. Male Wistar rats were fed commercial rat chow (CRF 1; Oriental Yeast Co., Tokyo, Japan) before surgery, during recovery, and between experiments. Rats were prepared with catheters draining pancreatic juice and bile separately and with a duodenal catheter. The operative procedures used have been described in detail in previous publications (5, 12). Briefly, after Enflurane anesthesia (Abbott, North Chicago, IL) was delivered to rats through a plastic face mask by means of a vaporizer, a midline incision was made to expose the duodenum. A catheter (0.025 in i.d. × 0.037 in o.d.; Silastic Medical Grade Tubing, Dow-Corning, Midland, MI) was inserted into the common bile duct proximal to the ampulla of Vater. Then, the common bile duct was ligated proximal to the pancreas near the liver, and the second catheter was inserted above the ligation below the bifurcation of the bile duct. An additional catheter was inserted into the duodenum to return BPJ. Finally, catheters were inserted into both the right and left jugular veins, the first for the continuous infusion of CR-1409 and the second for drawing blood samples. After the operation, rats were placed in modified Bollman-type restraint cages and had free access to food and water, in a 24°C room with filtered air, and light was scheduled from 05:00 through 17:00.

Experiments were conducted after 5 hr of fasting on the third and fourth postoperative days. During recovery, BPJ was continuously returned to the intestine via a duodenal cannula by a servo-system consisting of a collection tube in a liquid-level photodetector coupled to a peristaltic pump (5, 12). All rats were sacrificed for obtaining plasma samples to measure plasma CCK concentrations before (basal state) or after the diversion of various intervals (1, 2, 4, and 5.5 hr). Changes of pancreatic secretions, illustrated in Figures 1 (upper panel), to 3, were the results obtained from rats with 5.5 hr of diversion treatment.

Experimental Design. Bile and PJ were separately collected every 30-min period. The volume of PJ was measured by a Hamilton syringe and a 20-μl sample was taken for measuring protein and bicarbonate concentrations. Bile was collected in Wintrobe's hematocrit tubing, its volume being measured and mixed with the rest of the PJ. In order to obtain the basal secretion, bile and the rest of PJ were returned to the duodenum at a uniform rate by a syringe pump (Harvard Apparatus compact infusion pump; Harvard Apparatus, Southnatick, MA) during the next 30-min collection. After the 90-min collection of basal secretion with BPJ returning, BPJ, bile, or PJ was diverted from the intestine during which 0.05 *M* $NaHCO_3$ solution was infused at a rate of 1 ml/hr instead of each diverted component. Rats were sacrificed before diversion, at 1, 2, 4, and 5.5 hr after each diversion treatment. A 5-ml blood sample was drawn from the jugular vein cannula by a heparinized syringe. Blood samples were centrifuged at 3000 rpm for 15 min at 4°C for obtaining plasma. Plasma samples were kept at −70°C until the CCK bioassay.

To examine the participation of CCK during BPJ diversion, the intravenous infusion of CR-1409, one of CCK receptor antagonists, was started at a total dose of 0.1 mg/rat 30 min before BPJ diversion and following 2.5 hr during the diversion (13). CR-1409 was dissolved in 1% bovine serum albumin-saline solution.

Laboratory Assays. Bicarbonate concentration was measured by a Natelson microgasometer using a 10-μl sample immediately after each collection. Protein concentration was estimated by determining optical density at 280 nm of samples diluted 1/200 times in 0.04 *M* Tris buffer, pH 7.8 (14).

Bioassay of Plasma Cholecystokinin. Plasma CCK concentrations were measured by a bioassay using dispersed pancreatic acini as described by Liddle *et al.* (8) and modified by Louie *et al.* (9). CCK was extracted from 1 to 6 ml of rat plasma by adsorption onto C-18 SEP-PAK cartridges, which were previously washed with 10 ml of acetonitrile and 10 ml of methanol, followed with 40 ml of water. Then, the cartridges were washed again with 20 ml of water. The CCK was eluted with 1 ml of acetonitrile:water (1:1, v/v) into a polyethylene scintillation vial and dried in a 45°C water bath under a flow of nitrogen.

Isolated rat pancreatic acini were prepared by collagenase digestion of pancreas (15, 16) from fasted, ovariectomized female SD rats as described previously. Briefly, pancreatic tissue was digested by Krebs-Henseleit bicarbonate buffer containing 0.15 mg/ml purified collagenase and 1.8 mg/ml hyaluronidase, and then dispersed with forceful pipetting. After preincubation in Tris-Ringer solution, which contained 40 m*M* Tris as buffer, 0.5% bovine serum albumin, Eagle's minimum amino acids, and gassed with 100% O_2, 1-ml aliquots of acini suspension were added to the vials containing the plasma extracts or various concentrations of CCK-8 and incubated for 30 min at 37°C. Amylase released into the medium and total acinar amylase contents were measured by using the blue starch polymer as a substrate (17). Amylase released was expressed as a percentage of total amylase content of acini. Values were compared with the dose-response curve for CCK-8 and results were expressed as CCK-8 equivalents.

Statistics. Results of pancreatic secretions were analyzed by multiple analysis of variance with repeated measures with respect to treatments and time, followed by the Duncan's multiple range test. One-way analysis of variance, followed by the Newman-Keul's multiple comparison test, was used for analyzing the results of plasma CCK changes (18). Differences were considered

to be significant at $P < 0.05$. Values were expressed as means ± SE.

Results

Effects of Exclusion of BPJ, PJ, or Bile on Pancreatic Secretion and CCK Release. There were significant differences by multiple analysis of variance among animals given different treatments in fluid, bicarbonate, and protein outputs [$F(2, 15) = 6.87$ ($P < 0.01$) for fluid, 3.76 ($P < 0.05$) for bicarbonate, and 10.3 ($P < 0.001$) for protein outputs] (Figs. 1–3). Pancreatic protein secretion in response to BPJ diversion was significantly enhanced as compared with bile or PJ diversion during the first 2.5 hr of the 5.5-hr diversion period by multiple range test (Fig. 1). The diversion of BPJ caused an immediate and significant augmentation of pancreatic juice flow, bicarbonate and protein outputs, and a significant increase in plasma CCK concentrations (Figs. 1–3). F values with respect to time are depicted in the figure legends. The protein output and plasma CCK concentration reached their maxima during 1.5–2 hr and declined in the following 1.5–2 hr, but

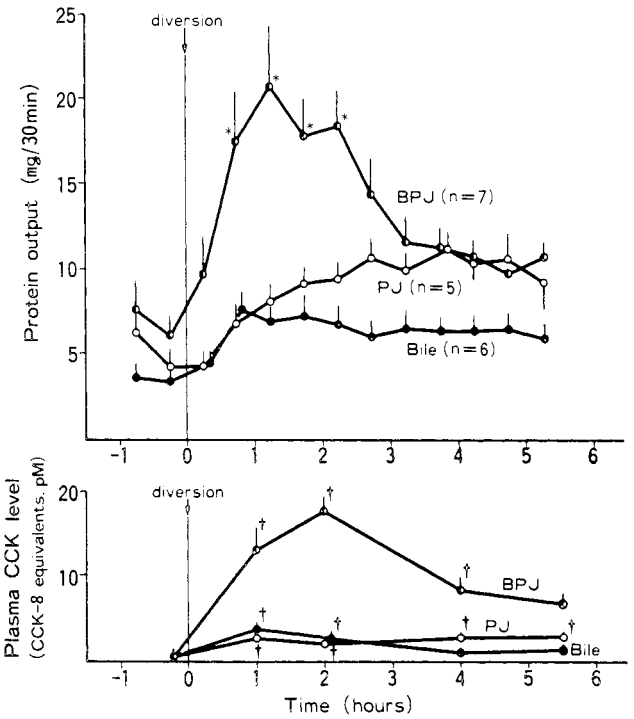

Figure 1. Pancreatic protein secretion (upper panel) and plasma CCK concentrations (lower panel) induced by diversion of bile, PJ, or BPJ. After 90 min of basal collection, bile, PJ, or BPJ was diverted from the duodenum. Changes in protein output by BPJ and PJ diversions were significant with respect to time ($F = 9.8$ and 7.1, respectively) but that by bile diversion was not significant ($F = 1.77$, $P > 0.2$). Plasma CCK levels which were bioassayed by using dispersed rat pancreatic acini significantly increased after diversions. Asterisks in the upper panel indicate significant differences from other two corresponding values by multiple range test. Daggers in the lower panel indicate significant differences from the basal value, 0.46 ± 0.05 pM by analysis of variance followed by multiple comparison test, $P < 0.05$ ($F = 14.97$ for BPJ diversion, $F = 4.86$ for PJ diversion and $F = 18.65$ for bile diversion).

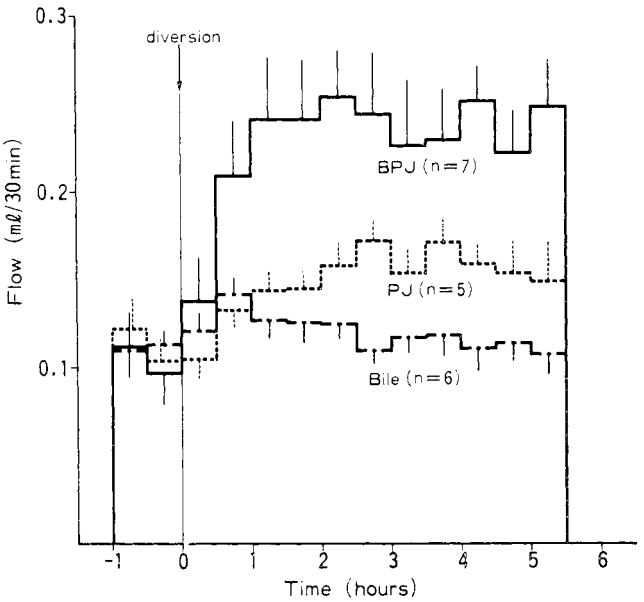

Figure 2. Pancreatic juice flow induced by diversion of bile, PJ, or BPJ. The experimental method is the same as that described in the legend of Figure 1. Changes produced by BPJ and PJ diversions with respect to time were significant ($F = 24.3$ and 11.5, respectively) but that by bile diversion was not significant ($F = 4.38$, $P > 0.05$).

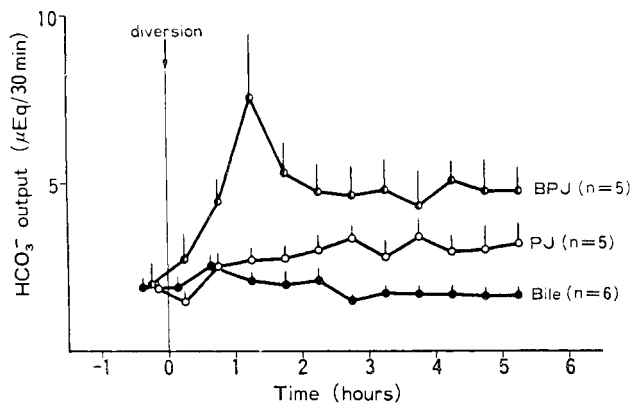

Figure 3. Pancreatic bicarbonate secretion induced by diversion of bile, PJ, or BPJ. The experimental method is the same as that described in the legend of Figure 1. Changes produced by BPJ and PJ diversion with respect to time were significant ($F = 20.4$ and 8.97, respectively) but that by bile diversion was not significant ($F = 2.45$, $P > 0.1$).

still remained elevated until the end of the experiment in comparison to each basal level.

PJ diversion caused gradual increases in fluid, bicarbonate, and protein outputs, and these remained elevated until 5.5 hr. Changes in pancreatic secretions with respect to time were all significant (F values are depicted in the legends of Figs. 1–3). Plasma CCK concentrations during PJ diversion were significantly higher than the basal value, 0.46 ± 0.05 pM (Fig. 1).

Bile diversion produced a smaller increase in protein output compared with BPJ diversion, without significant effects on both fluid and bicarbonate outputs by multiple analysis of variance (Figs. 1–3). The protein output slightly elevated in comparison to the basal value

until the end of the experiment, but the change was not statistically significant. Plasma CCK concentrations significantly increased for the first 2 hr after bile diversion, declining thereafter to the average level of 1.4 ± 0.6 p*M* at 5.5 hr (Fig. 1).

Plasma CCK levels induced by BPJ diversion at 1, 2, and 4 hr were greater than the sum of CCK levels produced by the two individual diversions of bile and PJ (Fig. 4).

Effect of CR-1409 Infusion on Pancreatic Secretion during BPJ Diversion. Pancreatic juice flow (Fig. 5, left panel) and protein output (Fig. 5, middle panel) were significantly inhibited when CR-1409 (0.1 mg/rat) was continuously infused during BPJ diversion [$F(1, 10) = 4.29$, $P < 0.05$ for fluid and $F(1, 10) = 5.8$, $P < 0.05$ for protein outputs], and they returned to basal levels after 2.5 hr. Net increases during 2.5 hr of BPJ diversion with or without CR-1409 infusion were calculated by subtracting values during 30 min before BPJ diversion, which was multiplied five times, from values during 2.5 hr after the diversion in each rat. Net increases of fluid and protein secretions decreased to 0.070 ± 0.103 ml and 10.62 ± 6.73 mg for 2.5 hr of BPJ diversion with CR-1409 infusion from respective control values (0.598 ± 0.082 ml and 53.84 ± 7.94 mg for 2.5 hr of BPJ diversion only). The net increase of bicarbonate secretion was slightly inhibited by CR-1409 (6.20 ± 3.62 μEq/2.5 hr with CR-1409 vs 14.98 ± 1.93 μEq/2.5 hr without CR-1409) (Fig. 5, right panel), but the difference was not statistically significant [$F(1, 10) = 1.82$, $P > 0.2$].

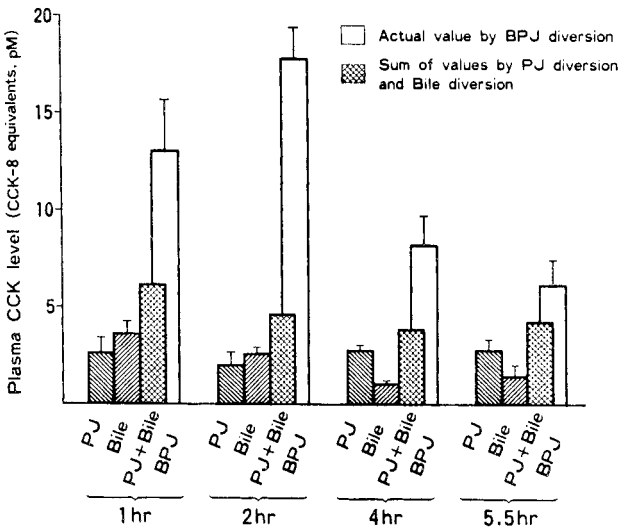

Figure 4. Plasma CCK levels induced by bile, PJ, or BPJ diversion. Bars represent means ± SE.

Discussion

We confirmed in this study that the exclusion of bile, PJ, of BPJ could stimulate not only pancreatic exocrine secretion but also CCK release (1, 5, 7, 11, 19), although the stimulatory effect of the simple bile diversion was weak. Among these treatments, BPJ diversion was the most potent stimulant to release endogenous CCK (Fig. 4). Values of plasma CCK concentration produced by BPJ diversion are comparable to those reported by Louie *et al.* (9). Furthermore, BPJ diversion also produced the higher protein secretion during the first 2-hr period of the diversion treatment as compared with PJ or bile diversion (Fig. 1). Meanwhile, protein

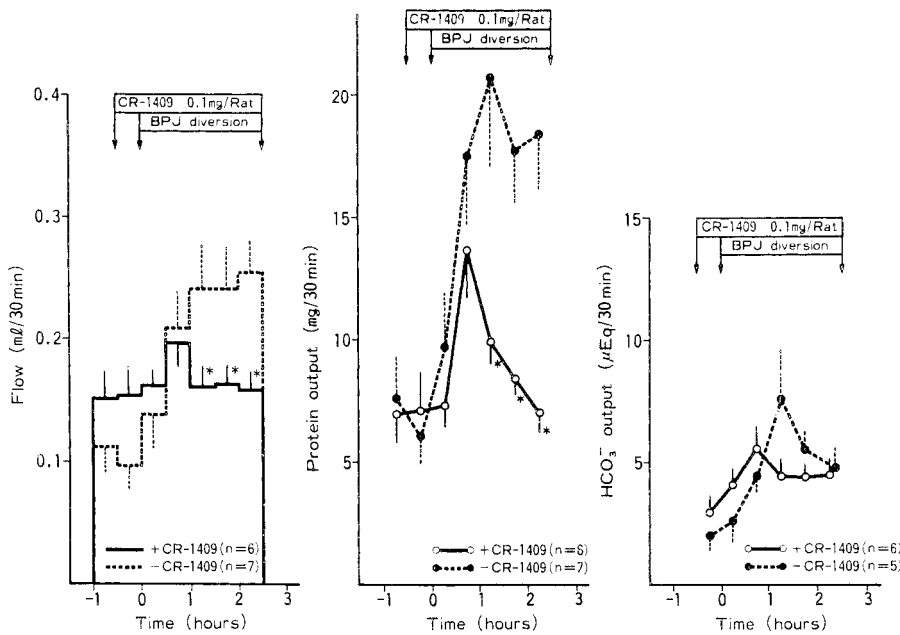

Figure 5. Inhibitory effect of intravenous infusion of CR-1409 on pancreatic secretion during BPJ diversion. The infusion of CR-1409 was started 30 min before BPJ diversion and continued thereafter for 2.5 hr at a total dose of 0.1 mg/rat. *F* values with respect to time were 1.48; $P > 0.3$ for fluid output (left panel), 6.18; $P < 0.05$ for bicarbonate output (right panel) and 5.5; $P < 0.05$ for protein output (middle panel). Asterisks indicate significant differences by multiple range test from corresponding values without CR-1409 infusion.

output (per 30 min) was almost the same between BPJ and PJ diversion during the last 2.5 hr of 5.5-hr diversion period (Fig. 1), while plasma CCK level was still significantly higher in BPJ diversion than in PJ diversion. Thus, it could be concluded that pancreatic protein secretion could not always correlate with plasma CCK concentration. The mechanism of this discrepancy between the plasma CCK level and protein secretion has been unknown. The desensitization of pancreatic acinar cells might have occurred due to the high level of plasma CCK released during early phase of BPJ diversion (20), although we could not exclude the possibility of participation of inhibitory hormones such as somatostatin.

It is surprising that bile or PJ diversion could produce at most 2–3 p*M* of CCK concentrations (Fig. 4) and the increase in plasma CCK concentration induced by BPJ diversion was much greater than can be explained simply as an additive effect of exclusions of PJ and bile especially during the first 2 hr of diversion period. That is, the responses to combined diversion of PJ and bile were far greater than the sum of effects of the separate diversions (Fig. 4). This result suggests that the combination of bile and pancreatic juice in the lumen might be more potent in inhibiting CCK release than the sum of effects of separate addition of these agents into the intestine. One of the possible mechanisms might be the stabilization of pancreatic proteases by bile acids (10, 11); however, other interactions between bile and PJ have not been elucidated.

One of CCK receptor antagonists, CR-1409, partially but significantly inhibited pancreatic fluid and protein secretions stimulated by BPJ diversion when infused at a dose of 0.1 mg/rat. These observations suggest that the pancreatic secretion increased by luminal feedback regulation in the conscious rat is mainly modulated by CCK as proposed in other studies (7, 9).

The exclusion of bile, PJ, or BPJ from the duodenum could stimulate CCK release and the diversion of PJ or BPJ could also stimulate pancreatic exocrine secretion. The apparent potentiation between bile and PJ diversions on CCK release was observed, and BPJ diversion was the most potent endogenous stimulant among the three in the conscious rat. It was also confirmed that CCK was a modulator of luminal feedback regulation.

1. Green GM, Lyman RL. Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats. Proc Soc Exp Biol Med **140**:6–12, 1972.
2. Green GM, Olds BA, Matthews G, Lyman RL. Protein, as a regulator of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med **142**:1162–1167, 1973.
3. Lyman RL, Olds BA, Green GM. Chymotrypsinogen in the intestine of rats fed soybean trypsin inhibitor and its inability to suppress pancreatic enzyme secretions. J Nutr **104**:105–110, 1974.
4. Schneeman BO, Lyman RL. Factors involved in the intestinal feedback regulation of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med **148**:897–903, 1975.
5. Miyasaka K, Green GM. Effect of partial exclusion of pancreatic juice on rat basal pancreatic secretion. Gastroenterology **86**:114–119, 1984.
6. Brand SJ, Morgan RGH. The release of rat intestinal cholecystokinin after oral trypsin inhibitor measured by bio-assay. J Physiol **319**:325–431, 1981.
7. Fölsch UR, Cantor P, Wilms HM, Schafmayer A, Becker HD, Creutzfeldt W. Role of cholecystokinin in the negative feedback control of pancreatic enzyme secretion in conscious rats. Gastroenterology **92**:449–458, 1987.
8. Liddle RA, Goldfine ID, Williams JA. Bioassay of plasma cholecystokinin in rats: Effects of food, trypsin inhibitor, and alcohol. Gastroenterology **87**:542–549, 1984.
9. Louie DS, May D, Miller P, Owyang C. Cholecystokinin mediates feedback regulation of pancreatic enzyme secretion in rats. Am J Physiol **250**:252–259, 1986.
10. Green GM, Nasset ES. Effect of bile duct obstruction on pancreatic enzyme secretion and intestinal proteolytic enzyme activity in the rat. Dig Dis Sci **22**:437–444, 1977.
11. Green GM, Nasset ES. Importance of bile in regulation of intraluminal proteolytic enzyme activities in the rat. Gastroenterology **79**:695–702, 1980.
12. Miyasaka K, Kitani K, Green GM. The sequential changes in pancreatic exocrine function after abdominal surgery in the rat. Pancreas **1**:347–353, 1986.
13. Miyasaka K, Nakamura R, Funakoshi A, Kitani K. Inhibitory effect of CR-1409, a competitive inhibitor of cholecystokinin, on pancreatic exocrine secretion in the conscious rat. Tohoku J Exp Med **155**:165–172, 1988.
14. Keller PJ, Cohen E, Neurath H. The proteins of bovine pancreatic juice. J Biol Chem **233**:344–349, 1958.
15. Otsuki M, Williams JA. Effect of diabetes mellitus on the regulation of enzyme secretion by isolated rat pancreatic acini. J Clin Invest **70**:148–156, 1982.
16. Williams JA, Korc M, Dormer RL. Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol **235**:517–524, 1978.
17. Ceska M, Birath K, Brown B. A new and rapid method for the clinical determination of α-amylase activities in human serum and urine. Optimal conditions. Clin Chim Acta **26**:437–444, 1969.
18. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ Res **47**:1–9, 1980.
19. Shiratori K, Chen YF, Chey WY, Lee KY, Chang TM. Mechanism of increased exocrine pancreatic secretion in pancreatic juice diverted rats. Gastroenterology **91**:1171–1178, 1986.
20. Abdelmoumene S, Gardner JD. Cholecystokinin-induced desensitization of enzyme secretion in dispersed acini from guinea pig pancreas. Am J Physiol **239**:272–279, 1980.