Secretory Capacity of Pancreatic Protein during Luminal Feedback Regulation in Conscious Rats

Kenji Okubo*1,*2, Masao Masuda*1, Kyoko Miyasaka*1,*3, and Akihiro Funakoshi*4

*1 Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo, 173 Japan
*2 Third Department of Internal Medicine, Yokohama City University School of Medicine, Yokohama, 236 Japan
** Division of Gastroenterology, National Kyushu Cancer Center, Fukuoka, 815 Japan

Abstract Pancreatic exocrine secretion in conscious rats is regulated by bile and pancreatic juice in the proximal intestine (luminal feedback regulation), and bile-pancreatic juice diversion from the intestine results in cholecystokinin (CCK) release and pancreatic hypersecretion. Pancreatic protein secretion increases to a maximum 60–90 min after bile-pancreatic juice diversion, and then decreases slightly to a steady level of two times the basal level. Change in plasma CCK concentration parallels protein secretion. In this study, the mechanism of the decreases of protein secretion and CCK concentration was examined by stimulation with various species of peptides having different stimulatory mechanisms. Cannulae for draining bile and pancreatic juice separately and a duodenal cannula and extrajugular vein cannula were inserted into male Wistar rats. Four days later, basal levels in a 90-min period were determined, bile and pancreatic juice were diverted for 90 min, and then either secretin (1.2 nmol/kg/h), CCK-8 (25 and 100 pmol/kg/h), neuromedin C (350 pmol/kg/h and 3.5 nmol/kg/h), or CCK-JMV-180 (200 nmol/kg/h) was infused intravenously for 60 min. Infusion of secretin significantly increased protein secretion and prevented its decrease after its maximum induced by bile-pancreatic juice diversion. The plasma CCK concentrations were not increased further by neuromedin C. In conclusion, pancreatic exocrine secretion and CCK release in conscious rats are maximally stimulated by luminal feedback regulation that the decrease after maximal protein output may be due to limitation of secretory capacity and/or desensitization of acinar cells.

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*3 To whom correspondence should be addressed.
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Pancreatic exocrine secretion in rats is regulated by bile and pancreatic juice in the proximal intestine (luminal feedback regulation) [1-4]. When bile and pancreatic juice are diverted from the intestine, protein output promptly increases to a maximum after 60-90 min, and then decreases to a steady level of twice the basal value (bile and pancreatic juice returned), despite continued bile-pancreatic juice diversion [4]. The changes in plasma cholecystokinin (CCK) concentrations parallel those of pancreatic protein output [4, 5]. Therefore, the decrease of protein secretion after bile-pancreatic juice diversion is considered to be due to a decrease in CCK release and/or a decrease in protein secretion by the pancreas. Another possibility is that peptides with inhibitory actions on pancreatic secretion, such as pancreatic polypeptide (PP) or peptide YY (PYY), are involved. However, changes in the plasma PP and PYY concentrations after the beginning of bile-pancreatic juice diversion almost parallel that of protein output [6, 7]. Thus, it was unlikely that these peptides are responsible for the decrease in protein secretion seen after its maximum. As reported previously [8] that bile-pancreatic juice diversion increases rather than decreases the intestinal CCK content, the later decrease in plasma CCK concentration during bile-pancreatic juice diversion could not be due to depletion of the CCK pool.

There are several possible explanations for the later decrease of protein secretion by pancreatic acinar cells, such as reduction of the secretory protein pool, desensitization of the cells to the high plasma CCK level, and the participation of a low affinity CCK receptor. Pancreatic acinar cells have been shown to respond to at least two different classes of secretagogues, causing intracellular calcium mobilization and activating adenylate cyclase [9]. To determine whether the later decrease of protein secretion is due to limitation of acinar cell function, we examined the effects of CCK-8 and neuromedin C, whose stimulatory actions are mediated by increase in intracellular calcium concentration, and of secretin, whose effect is mediated by the different mechanism of increase in cAMP concentration, on pancreatic secretion from 90 to 150 min after the beginning of bile-pancreatic juice diversion. CCK receptors of pancreatic acinar cells can be classified functionally into high- and low-affinity CCK receptors and that activation of high-affinity receptors results in hypersecretion of enzymes, while activation of low-affinity receptors causes inhibition of enzyme secretion [10-14]. In addition, to determine whether the decrease is mediated by the low-affinity CCK receptor, we infused CCK-JMV-180, an agonist of the high-affinity CCK receptor and antagonist of the low-affinity CCK receptor [10, 11], continuously before bile-pancreatic juice diversion or during the last 60 min of bile-pancreatic juice diversion. Finally, to determine whether the plasma CCK concentration could be restored to the peak level observed during 60-90 min of bile-pancreatic juice diversion, we infused
neuromedin C intravenously because gastrin-releasing peptide (GRP) families are reported to stimulate CCK release [15–17].

MATERIALS AND METHODS

**Materials and chemicals.** Synthetic CCK-octapeptide sulfate (CCK-8), CCK-33, and neuromedin C were purchased from the Peptide Institute, Osaka, Japan. CCK-JMV-180 was from Research Plus, Bayonne, New Jersey and porcine secretin was from Eisai, Tokyo, Japan. These peptides were dissolved in 1% bovine serum albumin (BSA) purchased from Sigma Chemicals, St. Louis, Missouri. The cannulae used in this study were Silastic Medical Grade Tubing (Dow-Corning, Midland, MI; 0.025 in. inner diameter, 0.037 in. outer diameter).

**Animal preparation.** Male Wistar rats (314–330 g) were obtained from Shizuoka Jikken Dobutsu (Shizuoka, Japan), and fed commercial rat chow (CRF-1, Oriental, Tokyo, Japan) before surgery and during recovery. After intraperitoneal anesthesia with sodium pentobarbital (15 mg/300 g body weight), a midline abdominal incision was made. A cannula 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 a second cannula was inserted above the ligation below the bifurcation of the bile duct. Thus, pure bile and pure pancreatic juice were collected separately. A third cannula was inserted into the duodenum to return bile and pancreatic juice, its outlet tip being located near the ampulla of Vater. All cannulae were initially brought into the abdominal cavity through a subcutaneous channel in the back near the tail. Finally, a cannula was inserted into the right jugular vein. Details of the operative procedures and the maintenance of animals after surgery were as described previously [18, 19]. After the operation, the rats were placed in modified Bollman-type restraint cages, and given free access to food and water in a room at 24°C with filtered air and light from 5 AM through 5 PM. Bile and pancreatic juice were continuously returned to the intestine via the duodenal cannula. Experiments were conducted on day 4 after the operation after withholding food for 5 h [18].

**Experimental design.** Bile and pancreatic juice were collected separately for 30-min periods and the volume of pancreatic juice was measured with a Hamilton syringe. Samples of 15 and 10 μl of pancreatic juice were used for determining protein and bicarbonate concentrations, respectively, and the rest was mixed with the bile and infused into the duodenum with a syringe pump (Compact Infusion Pump, Harvard Apparatus, Southnatick, MA) over the next 30 min. The first 30-min sample was not used for assay, and during the first 30 min of the experiment the pooled bile and pancreatic juice were infused. After a basal collection period of 90 min with return of bile and pancreatic juice, the bile and pancreatic juice were diverted from the intestine and 0.05 M NaHCO₃ was infused instead of bile and pancreatic juice throughout the experiment. In control experiments, bile and pancreatic juice were divered after 90 min basal collection and changes of pancre-
atic secretion were examined for 2 or 2.5 h. Before bile-pancreatic juice diversion, 1, 2, and 2.5 h after diversion, 6 ml of blood was withdrawn through the jugular vein cannula into a heparinized syringe and the animals were sacrificed. Blood samples were collected in ice-chilled EDTA tubes and were immediately centrifuged at 4°C at 3,000 rpm for 15 min, and 3 ml of plasma was obtained from each rat. The plasma samples were stored at −70°C until assay.

Effects of graded doses of neuromedin C on pancreatic secretion and plasma CCK concentration during bile and pancreatic juice diversion. After 90 min of collection of pancreatic juice during bile-pancreatic juice diversion, 350 pmol/kg/h of neuromedin C was infused intravenously for 60 min (1 ml/h). This dose was considered to be a submaximal dose [20]. Changes of fluid, bicarbonate and protein secretions were examined and the plasma CCK concentration was measured at the end of the experiment.

For examination of the effect of neuromedin C on CCK release, blood was withdrawn from some animals 30 min after the beginning of intravenous infusion of neuromedin C (350 pmol/kg/h or 3.5 nmol/kg/h) because the stimulatory effect of neuromedin C on CCK release was transient [17].

Effects of graded doses of CCK-8 on pancreatic secretion and plasma CCK concentration during bile and pancreatic juice diversion. After 90 min of collection of pancreatic juice during bile-pancreatic juice diversion, CCK-8 (25 or 100 pmol/kg/h) was infused for 60 min. Changes of fluid and protein secretions were examined and the plasma CCK concentration was measured at the end of experiment in which 100 pmol/kg/h of CCK-8 was infused.

Effects of graded doses of secretin on pancreatic secretion. Pancreatic response to graded doses of secretin: Secretin has been known to act on duct cells and stimulate fluid and bicarbonate secretion. When a larger dose such as 10−7 M was applied, secretin stimulated amylase release from dispersed acinar cells, in vitro. To examine the optimal dose of secretin to stimulate protein secretion in vivo, secretin (21.7 or 65.0 pmol/kg/h, or 1.2 nmol/kg/h) was infused for 120 min during bile and pancreatic juice return. We found that infusion of 1.2 nmol/kg/h of secretin could produce sustained protein hypersecretion.

Effects of secretin on pancreatic secretion and plasma CCK concentration during bile and pancreatic juice diversion: Bile and pancreatic juice were diverted as described above. After 90 min of collection of pancreatic juice during bile-pancreatic juice diversion, 1.2 nmol/kg/h of secretin was infused intravenously for 60 min. Changes of fluid, bicarbonate, and protein secretions were examined and plasma CCK concentrations were examined.

Effects of graded doses of CCK-JMV-180 on pancreatic secretion. Pancreatic response to graded doses of CCK-JMV-180: After 90 min of basal secretion with bile and pancreatic juice return, graded doses of CCK-JMV-180 (8, 40, or 200 nmol/kg/h) were infused intravenously for 150 min. Bile and pancreatic juice were continuously returned to the proximal intestine throughout the experiment.

Effect of the maximal dose of CCK-JMV-180 on pancreatic secretion during
bile and pancreatic juice diversion: The experimental design was similar to that for neuromedin C, CCK-8, and secretin. After 90 min of bile-pancreatic juice diversion, 200 nmol/kg/h of CCK-JMV-180 was infused intravenously for 60 min.

To examine whether CCK-JMV-180 prevented the later decrease of pancreatic secretion, 40 nmol/kg/h of CCK-JMV-180 was infused into some animals from 30 min before bile-pancreatic juice diversion, and bile-pancreatic juice diversion was continued for 150 min.

**Assays.** The plasma CCK concentration was measured by radioimmunoassay (RIA) using antiserum OAL-656 (Otsuka Assay Laboratories, Tokushima, Japan) and CCK-8 as a standard [21, 22]. [125I] Porcine CCK-39, prepared by the Iodogen method, was used as a tracer after its purification on an SP-Sephadex column (Pharmacia LKB Uppsala, Sweden). The antiserum reacted specifically with an amino-terminal region of CCK-8, and bound 100% of CCK-8 and CCK-33 and 85% of CCK-39, but did not cross-react with the nonsulfated form of CCK-8. The sensitivity of the assay was 1.3 fmol/tube, equivalent to 6.6 pm as CCK-8.

CCK was extracted from 2–3 ml of plasma by adsorption to a C-18 column as described elsewhere [23]. For measurement of basal plasma CCK, rats were sacrificed after 2 h of bile and pancreatic juice return and two 3-ml plasma samples were mixed to obtain a sample of 6 ml.

Protein concentration in pancreatic juice was determined by measuring the optical density at 280 nm [24] of samples diluted 200-fold with 0.04 M Tris buffer at pH 7.8. Bicarbonate concentration was determined by a Natelson microgasometer. Protein and bicarbonate outputs were estimated.

**Statistical analysis.** Values are expressed as means ± SE. Changes in pancreatic secretion were analyzed by the unpaired t-test. Changes in plasma CCK concentrations and increases of pancreatic secretions were analyzed by one-way analysis of variance (ANOVA) with respect to treatment, followed by Newman-Keul's multiple comparison test. A value of p < 0.05 was considered significant.

**RESULTS**

_Effects of neuromedin C on pancreatic secretion and plasma CCK concentrations during bile-pancreatic juice diversion_

Diversion of bile and pancreatic juice from the intestine strongly stimulated pancreatic secretion and plasma CCK concentrations (Fig. 1, open circles). Protein output increased significantly to a peak after 60–90 min and then decreased slightly to 2–3 times the basal value, whereas the increase in fluid secretion remained constant. Bicarbonate output also increased slightly but significantly. The plasma CCK concentration was significantly increased by bile and pancreatic juice diversion, peaked at 60–120 min after diversion and declined slightly. The plasma CCK concentration in control rats (bile and pancreatic juice return) was 3.6 ± 1.9 pm (mean ± SE for 10 rats in 5 assay samples).
Fig. 1. Changes of pancreatic secretion and plasma CCK concentrations during bile-pancreatic juice diversion with and without infusion of 350 pmol/kg/h of neuromedin C (NC). Bile-pancreatic juice (BPJ) was diverted for 2.5 h and 350 pmol/kg/h of neuromedin C was infused intravenously for 60 min. The changes of pancreatic secretion in terms of secretion of fluid (A), bicarbonate (B), and protein (C) were similar to those produced by simple diversion of bile and pancreatic juice diversion. n = 6 with neuromedin C and 7 for simple bile-pancreatic juice diversion. The plasma CCK concentrations were measured before diversion, 1, 2, 2.5 h after diversion. For measuring plasma CCK, 4–10 rats were sacrificed at each time point. All values after bile-pancreatic juice diversion were significantly different from the basal value but NC did not affect plasma CCK concentrations.

Intravenous infusion of neuromedin C (350 pmol/kg/h) did not affect the changes in pancreatic secretions in response to bile-pancreatic juice diversion (Fig. 1, closed circles). The increases of protein secretion during 60 min of peptide infusion, calculated as the values after 60 min peptide infusion minus the values 60 min before peptide infusion, are shown in Fig. 2. There was no significant
difference between the values on bile-pancreatic juice diversion with and without infusion of neuromedin C.

Infusion of neuromedin C for 30 min, even at a high dose, did not increase the plasma CCK concentrations further (Fig. 1D).

**Effects of graded doses of CCK-8 on pancreatic secretion and plasma CCK concentration during bile and pancreatic juice diversion**

Intravenous infusion of CCK-8 (25 or 100 pmol/kg/h) decreased fluid secretion stimulated by bile-pancreatic juice diversion, but increased the protein concentration, and so did not change protein output. The effect of infusion of 100 pmol/kg/h of CCK is shown in Figs. 2 and 3.

The plasma CCK concentration after infusion of 100 pmol/kg/h of CCK-8 was 575.3 ± 53.5 pm (mean ± SE).

**Effects of graded doses of secretin on pancreatic secretion**

**Effects of graded doses of secretin on pancreatic secretion during bile-pancreatic juice return.** Intravenous infusion of secretin, except the lowest dose (27.5 pmol/kg/h), significantly stimulated pancreatic fluid, bicarbonate, and protein secretion...
Fig. 3. Changes of pancreatic secretion during bile-pancreatic juice diversion with and without infusion of 100 pmol/kg/h of CCK. Infusion of CCK decreased fluid secretion but not protein output. \( n = 7 \) for each treatment.

in a dose-related manner (Fig. 4). Since secretin 1.2 nmol/kg/h produced a sustained hypersecretion of protein, we chose this dose to examine the effect on the later decrease of protein secretion produced by bile-pancreatic juice diversion.

Effects of graded doses of secretin on pancreatic secretion and plasma CCK concentration during bile and pancreatic juice diversion. Intravenous infusion of 1.2 nmol/kg/h of secretin caused significant increases in fluid, bicarbonate, and protein concentration, and consequently an increase in protein output (Fig. 5).

The plasma CCK concentration was not affected by infusion of secretin (32.7 ± 1.9 pm 2.5 h after bile-pancreatic juice diversion alone vs. 32.8 ± 4.0 for bile-pancreatic juice diversion with 60-min infusion of secretin).

Effect of CCK-JMV-180

Effect of graded doses of CCK-JMV-180 on pancreatic secretion during bile and pancreatic juice return. CCK-JMV-180 stimulated pancreatic protein secretion in a dose-related manner, although its effect on fluid secretion was small and not significant (Fig. 6A). Because 40 nmol/kg/h of CCK-JMV-180 stimulated pancreatic protein output continuously, we considered that this dose was the most effective and the maximal dose for conscious rats. Infusion of CCK-JMV-180 at 200 nmol/kg/h increased protein output more than infusion at 40 nmol/kg/h in the first 1 h of infusion, but resulted in a decrease in protein output, thereafter.

Effect of graded doses of CCK-JMV-180 on pancreatic secretion during bile and pancreatic juice diversion. Like neuromedin C and CCK-8, CCK-JMV-180 did not affect protein secretion during bile-pancreatic juice diversion. Its effect on protein secretion is shown in Fig. 2. Infusion of 40 nmol/kg/h of CCK-JMV-180 before bile-pancreatic juice diversion did not affect protein or fluid secretion or restore protein output (Fig. 6B).
Fig. 4. Changes of pancreatic secretion stimulated by graded doses of secretin during bile and pancreatic juice return. The lowest dose of secretin (27.5 pmol/kg/h) did not affect pancreatic secretion significantly, but higher doses did. n = 6 for each group.
Fig. 5. Changes of pancreatic responses during bile-pancreatic juice diversion with infusion of 1.2 nmol/kg/h of secretin (n = 6). Data shown as open circles were the same as shown in Fig. 1. Infusion of secretin significantly increased fluid, bicarbonate, and protein secretion, and no decline of protein secretion was observed during the last 60 min. *Significantly different from the values of bile-pancreatic juice diversion alone.
Fig. 6. Changes of pancreatic protein secretion stimulated by graded doses of CCK-JMV-180 during bile and pancreatic juice return. Protein outputs (A) during the last 2 h were significantly higher than the basal value with each dose of CCK-JMV-180. Dose of CCK-JMV-180: 8 nmol/kg/h (open circles, \( n = 5 \)), 40 nmol/kg/h (closed circles, \( n = 4 \)), and 200 nmol/kg/h (open squares, \( n = 5 \)). Changes of protein secretion on infusion of a submaximal dose of CCK-JMV-180 (40 nmol/kg/h) before bile-pancreatic juice diversion (B). The later decline in protein secretion was still observed. *Significantly higher than the basal value (\( n = 5 \)).

DISCUSSION

In this study, we tried to clarify the mechanism of the later decreases of protein secretion and CCK release produced by bile-pancreatic juice diversion. The results showed that 1.2 nmol/kg/h of secretin increased protein output and prevented this later decrease of pancreatic secretion produced by bile-pancreatic juice diversion (Figs. 2 and 5). However, CCK-8, neuromedin C, and CCK-JMV-180 did not affect the later decrease of protein secretion produced by bile-pancreatic juice diversion. This observation is similar to a phenomenon referred to as desensitization observed in *in vitro* experiments using dispersed pancreatic acinar cells [25, 26]. That is, prior incubation of acini with secretagogues whose actions are mediated by an increase in cellular calcium concentration reduced the subsequent stimulation of amylase release caused by these secretagogues [25–29]. Endogenously released CCK is thought to be the main factor modulating pancreatic secretion during bile-pancreatic juice diversion [1–4]. We previously examined changes of plasma CCK concentration by bioassay using dispersed acini 1, 2, and 4 h after bile-pancreatic juice diversion, and found that the plasma CCK concentration increased to 12.7 and 17.9 pm after 1 to 2 h, respectively, and then decreased, slightly to 7.6 pm after 4 h [4]. On the other hand, Liddle *et al.*, using the same assay as us, reported [30] that the plasma CCK concentration after food intake in
rats is about 6 pm which then decreases to a level of about 1 pm within 10 min. Therefore, stimulation by bile-pancreatic juice diversion may be unphysiologically potent in terms of the high level of plasma CCK concentration induced and the long duration of its effect; that is, the CCK release in response to bile-pancreatic juice diversion is far in excess of that necessary to stimulate maximal pancreatic protein secretion. In an in vitro experiment, Abdeloumene et al. reported [25] that CCK-induced desensitization occurred in a range of CCK concentrations that were supramaximal for stimulating amylase release. Thus, we suggest that a high plasma CCK level induced by bile-pancreatic juice diversion initially produces an increase of cellular calcium and subsequent desensitization of acinar cells to CCK analogues and neuromedin C, whose actions were mediated by cellular calcium. However, administration of 1.2 nmol/kg/h of secretin further increased fluid and bicarbonate outputs over peak secretions, but protein output was not further increased although the later decrease was prevented (Fig. 5). Therefore, the possibility that the secretory capacity of acinar cells were exhausted because of maximal stimulation could not be excluded. On the other hand, fluid secretion did not decrease during bile-pancreatic juice diversion. We did not measure changes of plasma secretin concentration using our models; however, secretin might be also increased during bile-pancreatic juice diversion.

In our in vivo study, CCK-JMV-180 stimulated pancreatic secretion in a dose-dependent manner, but the highest dose of CCK-JMV-180 tested produced a later decrease of protein secretion (Fig. 6A), similar to the phenomenon observed after bile-pancreatic juice diversion although Saluja et al. did not observe any decrease of protein secretion in vivo in anesthetized rats [31]. The reason for this difference between their results and ours is unknown. Prior stimulation with CCK-JMV-180, which antagonizes the low-affinity receptor, did not prevent the later decrease of protein secretion after bile-pancreatic juice diversion. Therefore, this later decrease does not seem to be attributable to an effect via the low affinity receptor for CCK.

We measured plasma CCK concentrations by RIA in this study. Although we have previously reported that the values of plasma CCK concentrations measured by RIA and bioassay were correlated [32], the values obtained by RIA were higher than those by bioassay [5]. However, regardless of bioassay or RIA, change in plasma CCK concentration paralleled the change of protein output during bile-pancreatic juice diversion with the plasma CCK concentration increasing to a peak 60–120 min after the beginning of bile-pancreatic juice diversion, and then decreasing slightly [4]. Neuromedin C, bombesin and GRP are reported to induce the release of CCK [15–17]. In a previous study [17], we found that neuromedin C stimulated pancreatic secretion in part via release of CCK, and that the plasma CCK level was increased after its infusion for 15 and 30 min at 350 pmol/kg/h. In the present study, we measured the plasma CCK levels after infusion of graded doses of neuromedin C for 30 min, but detected no further elevation of the plasma
CCK level over that after 2 h after bile-pancreatic juice diversion (Fig. 1).

It has been suggested that CCK release in conscious rats is regulated by luminal CCK-releasing-peptides [33–35] derived from pancreatic juice (named monitor peptide) and from the intestinal mucosa. In the present study, the contribution of monitor peptide could be excluded because bile and pancreatic juice were excluded from the intestinal lumen. CCK cells have a scattered distribution in the intestinal mucosa, so it is difficult to examine their functions. Recently, however, Liddle et al. succeeded in obtaining a preparation enriched in CCK cells and found that CCK was released through an increase in intracellular calcium after the binding of monitor peptide [36]. Taken together, these findings suggest that CCK releasing cells may become desensitized, although we could not demonstrate overcoming the reduced CCK release by using some other stimulant such as secretin on protein secretion.

We conclude that pancreatic exocrine secretion and CCK release in conscious rats are maximally stimulated by luminal feedback regulation and that the decrease after maximal protein output may be due to limitation of secretory capacity and/or desensitization of acinar cells and probably CCK-releasing cells.

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