Differential mechanism and site of action of CCK on the pancreatic secretion and growth in rats

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Recent studies have demonstrated that the vagal nerve plays an important role in digestive physiology, including the control of pancreatic functions (7, 22). In addition, both cholecystokinin (CCK)-1 and CCK-2 receptors are revealed to be present on rat and rabbit vagal fibers (25, 45). Although CCK has been shown to play an important physiological role both in the meal-induced release of pancreatic enzymes and in the regulation of pancreatic growth (10, 30, 33, 37), the influence of vagotomy on the pancreatic growth is still controversial (2, 5, 16, 26, 27, 39, 41).

Capsaicin, the pungent ingredient in red peppers, is a selective neurotoxin for unmyelinated primary afferent sensory neurons (36). It is therefore widely used as a pharmacological tool to assess the involvement of sensory neurons in biological functions. In addition, immunochemistry and retrograde tracing demonstrated the presence of capsaicin-sensitive afferent nerve fibers in the gastrointestinal mucosa and the pancreas (3). Although the mechanism and site of action of CCK on the pancreatic secretion is still controversial, Li and Owyang (20, 21) have revealed that exogenous and endogenous CCK at physiological concentrations stimulates pancreatic enzyme secretion via a capsaicin-sensitive afferent vagal pathway in anesthetized rats. A recent study also showed that vagal hyperactivity itself stimulates cell proliferation of pancreatic β and acinar cells primarily through a cholinergic receptor in rats (15). Along this line, we hypothesized that the ablation of afferent vagal fibers interrupts the effect of CCK on the pancreatic growth and results in atrophy or hypoplasia of the pancreas. In the present study, we examined our hypothesis and determined the site of action of CCK on pancreatic secretion and growth in rats.

MATERIALS AND METHODS

Materials

The synthetic trypsin inhibitor camostat (FOY-305) was supplied by Ono Pharmaceutical (Osaka, Japan). The following were also purchased: capsaicin, calf thymus DNA, enterokinase, and trypsin (type 1; 10,100 U/mg solid) (Sigma, St. Louis, MO); octapeptide of CCK (CCK-8) (Peptide Institute, Protein Research Foundation, Osaka, Japan); CCK-8 NH2-terminal-specific rabbit antiseraum OAL-656 (Otsuka Assay Laboratory, Tokushima, Japan); fluorescent dye H-33258 (Hoechst, Frankfurt, Germany); Phadebas amylose test (amylose test A) (Shionogi Pharmaceutical, Osaka, Japan); guanidine thiocyanate (Fluka Biochemika, Buchs, Switzerland); and guinea pig pancreas (Charles River, Gardiner, ME). A synthetic trypsin inhibitor, camostat mesylate, was synthesized by the Research Institute for Pharmaceutical Sciences, University of Shizuoka (Shizuoka, Japan). Pharmacological antiseraum OAL-656 was purchased from Otsuka Pharma. Co., Ltd. (Tokushima, Japan).

Methods

Experiments were performed on male Wistar rats (200–250 g) under deep anesthesia with urethane (1.3 g/kg i.p.) or sodium pentobarbital (30 mg/kg i.p.). Atrumatic cannula were inserted into the duodenum (jugular vein) for intravenous infusion or intraduodenal infusion of trypsin and pancreozymin and for continuous intraduodenal infusion of synthetic CCK-8 (CCK-8) and capsaicin. Details of mice experiments were described in a previous report (15). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Switzerland); nylon membranes (Hybond-N), polyvinylidene difluoride (PVDF) membranes (Hybond-P), and [α-32P]deoxyctydine triphosphate (Amersham Pharmacia Biotech, London, UK); and a random primer DNA labeling kit (version 2) (Takara Shuzo, Shiga, Japan).

Animals

Male Wistar rats weighing 200–220 g were used in the present study and maintained in a temperature (23 ± 2°C) and humidity (55 ± 5%)-controlled room with a 12:12-h light-dark cycle (lights on at 7:00 AM). Rats received humane care according to the guidelines of our institution, and the experimental protocol was approved by our institutional animal welfare committee.

Animal Preparation

For capsaicin treatment, rats were anesthetized with intraperitoneal injection of pentobarbital sodium at 50 mg/kg body wt. After a midline laparotomy, the abdominal vagal trunks were exposed, and a piece of gauze soaked in 0.1 ml capsaicin solution (10 mg/ml dissolved in Tween 80 and olive oil) was left on the vagal trunks for 30 min. Vehicle alone was applied to control rats. Experiments were performed on the seventh day after the treatment.

For continuous administration of trypsin (2 mg/h) into the duodenum after capsaicin treatment, rats were fasted overnight and anesthetized with pentobarbital sodium (50 mg/kg body wt ip), and a cannula was inserted into the duodenum. Rats were placed in Bollman-type restraint cages and were allowed to access food and water ad libitum after the surgery.

For pancreatic secretory study, rats were fasted overnight and anesthetized with pentobarbital sodium (50 mg/kg body wt ip). Two cannulas were inserted into the biliopancreatic duct and bile duct to drain pure pancreatic juice and pure bile separately. In addition, two cannulas were inserted into the duodenum to return bile pancreatic juice and infuse camostat. A jugular vein cannula was inserted for infusion of CCK-8.

Experimental Protocols

Food intake study. Since capsaicin application to central and peripheral vagal fibers attenuates CCK-induced satiety (38), food intake after infusion of CCK-8 was determined to demonstrate that perivagal capsaicin application successfully ablated vagal afferent fibers. On postoperative day 3, rats were fasted from 9:00 AM to 5:00 PM and then injected with CCK-8 (200 pmol/kg body wt ip). Five minutes later, rats were allowed to eat for 30 min, and food intake during this period was measured.

Effect of capsaicin treatment on pancreatic growth. To examine the effect of chemical ablation of afferent vagal fibers by capsaicin on pancreatic growth, the whole pancreas was removed and weighed without fasting on day 7 after the treatment. The whole pancreas was removed and weighed without fasting on day 7 after capsaicin treatment.
anesthetized rats (42). Pancreatic secretion was collected every 10 min, and fluid volume and protein concentration were determined. Blood was taken to determine the plasma CCK level at fasting and immediately after the end of camostat administration.

To examine the effect of endogenous CCK on pancreatic growth, camostat at a dose of 100 mg/kg body wt was given orally once daily for 7 days, and the whole pancreas was removed and weighed in the capsaicin-treated and control rats. Vehicle alone was administered orally once daily for 7 days in control rats.

**Site of action of exogenous CCK on pancreatic secretion and growth.** To examine the effect of exogenous CCK on pancreatic exocrine secretion and pancreatic growth in capsaicin-treated and control rats, a synthetic CCK-8 at 40 pmol·kg body wt⁻¹·h⁻¹ was infused via the jugular vein for 1 h. Pancreatic secretion was collected every 10 min and was analyzed for volume and protein. Blood was taken at the end of CCK-8 infusion to determine plasma CCK levels.

To examine the effect of exogenous CCK on pancreatic growth in capsaicin-treated and control rats, CCK-8 at 40 pmol/kg body wt was injected intraperitoneally twice daily (9:00 AM and 9:00 PM) for 7 days, and the whole pancreas was removed and weighed.

**Assays**

Protein concentrations in pancreatic homogenate and pancreatic juice were measured by the method of Lowry et al. (23) with bovine plasma albumin as a standard. Pancreatic DNA was measured fluorometrically by the reaction between 3, 5-diaminobenzoic acid and deoxyribose sugar by using calf thymus DNA as a standard (18). CCK concentrations in plasma were measured by a sensitive and specific radioimmunoassay using the antiserum OAL-656 with CCK-8 as a standard (40). Amylase activity was determined by a chromogenic method with Phadebas amylase test and expressed as a Somogyi unit. Trypsinogen was determined as trypsinogenic method with Phadebas amylase test and expressed as international units.

**Statistical Analysis**

Each experiment was performed in 7–9 rats, and results were expressed as means ± SE. Statistical analysis was performed by unpaired Student’s t-test using a commercial software, StatView (Abacus Concepts/Brain Power, Berkeley, CA). Differences of P < 0.05 were considered statistically significant.

**RESULTS**

**Effect of Capsaicin Treatment on CCK-8-Induced Inhibition of Food Intake**

CCK-8 had no influence on food intake in capsaicin-treated rats, whereas it significantly inhibited food intake in control rats (Table 1). These results indicate that the vagal afferent fibers mediating the satiety effect of CCK are functionally ablated after capsaicin treatment.

**Effect of Capsaicin Treatment on Pancreatic Growth**

Chemical ablation of the afferent vagal fibers by capsaicin had no influence on body weight gain, but it caused a significant increase in pancreatic wet weight compared with that in the control rats (Table 1). Pancreatic protein and DNA contents were also significantly increased after perivagal application of capsaicin, whereas the ratio of protein to DNA, an indication of cell size, remained unchanged in both groups (Table 1). These observations indicate that the increase in pancreatic wet weight after capsaicin treatment is due to hyperplasia.

**Effect of Capsaicin Treatment on Pancreatic Enzyme Contents**

Pancreatic amylase, lipase, and trypsinogen contents were increased in capsaicin-treated rats compared with those in control rats, although a statistically significant difference was found only in the trypsinogen content (Table 1). When pancreatic enzyme contents were related to pancreatic DNA, pancreatic amylase concentration showed no significant change, lipase concentration slightly decreased, and trypsinogen concentration significantly increased after capsaicin treatment (Table 1).

**Plasma CCK Levels and the Effect of Loxiglumide on Pancreatic Growth after Capsaicin Treatment**

Plasma CCK levels in fasting and nonfasting states in capsaicin-treated rats were higher than those in the control rats (Table 2). Oral administration of the
CCK-1 receptor antagonist loxiglumide prevented the increase in pancreatic wet weight in capsaicin-treated rats (Fig. 1). These results suggest that endogenous CCK plays an important role in the pancreatic growth in capsaicin-treated rats.

**Effect of Intraduodenal Infusion of Trypsin on Plasma CCK Levels and Pancreatic Growth after Capsaicin Treatment**

Continuous intraduodenal infusion of trypsin completely inhibited the elevation of plasma CCK levels in both fasting and nonfasting states in capsaicin-treated rats and in the nonfasting state in control rats (Table 2). The increase in pancreatic wet weight was also completely prevented by intraduodenal infusion of trypsin in capsaicin-treated rats (Fig. 1). These results suggest that the increase in plasma CCK levels after capsaicin treatment is due to the decrease of proteolytic activity in the duodenum.

### Table 2. Plasma CCK levels in control and capsaicin-treated rats

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<th>Control Rats</th>
<th>Capsaicin-Treated Rats</th>
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<tr>
<td>Fasting state</td>
<td>1.5 ± 0.2</td>
<td>3.7 ± 0.4*</td>
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<tr>
<td>+ infusion of trypsin</td>
<td>1.5 ± 0.1</td>
<td>1.7 ± 0.2†</td>
</tr>
<tr>
<td>Nonfasting state</td>
<td>5.3 ± 1.4</td>
<td>8.4 ± 2.0</td>
</tr>
<tr>
<td>+ infusion of trypsin</td>
<td>2.3 ± 0.3†</td>
<td>2.2 ± 0.3†</td>
</tr>
<tr>
<td>Administration of camostat</td>
<td>8.5 ± 2.0</td>
<td>10.7 ± 3.5</td>
</tr>
<tr>
<td>Infusion of CCK-8</td>
<td>10.2 ± 1.2</td>
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Values are means ± SE of 7–9 rats given as picmoles per liter. *Significant difference vs. control rats. †Significant difference vs. without trypsin in fasting and nonfasting states. Plasma was obtained after a 16-h fast, without fasting and at the end of the administration of camostat (100 mg·kg body wt⁻¹·h⁻¹) or CCK-8 (40 pmol·kg body wt⁻¹·h⁻¹) for 1 h.

CCK-1 receptor antagonist loxiglumide prevented the increase in pancreatic wet weight in capsaicin-treated rats (Fig. 1). These results suggest that endogenous CCK plays an important role in the pancreatic growth in capsaicin-treated rats.

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![Fig. 1. Effect of oral administration of cholecystokinin (CCK)-1 receptor antagonist loxiglumide and intraduodenal infusion of trypsin on pancreatic weight in capsaicin-treated rats. Values are expressed as means ± SE of 7–9 rats. *Significant difference vs. control rats.](image1)

**Expression Levels of CCK-1 Receptor mRNA and Protein in the Capsaicin-Treated and Control Rats**

There were no significant differences in the CCK-1 receptor mRNA and protein levels between capsaicin-treated and control rats (Fig. 2).

**Effect of Capsaicin Treatment on Pancreatic Exocrine Secretion and Growth in Response to Endogenous CCK Releaser Camostat**

Intraduodenal administration of camostat at a dose of 100 mg·kg body wt⁻¹·h⁻¹ produced a great increase in pancreatic protein and fluid secretion in control rats but not in capsaicin-treated rats (Fig. 3A), although pancreatic fluid in basal secretion was significantly higher in capsaicin-treated rats compared with that in control rats (Fig. 3B). Intraduodenal infusion of camostat elicited significant increases in plasma CCK levels above the basal values in both control and capsaicin-treated rats. There were no significant differences in plasma CCK levels between control and capsaicin-treated rats after camostat infusion (Table 2). Once-daily oral administration of 100 mg·kg body wt camostat for 7 days significantly increased pancreatic weight compared with that in vehicle-treated control rats, irrespective of whether vagal nerves were treated with capsaicin or not (Table 1).

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**Fig. 1.** Effect of oral administration of cholecystokinin (CCK)-1 receptor antagonist loxiglumide and intraduodenal infusion of trypsin on pancreatic weight in capsaicin-treated rats. Values are expressed as means ± SE of 7–9 rats. *Significant difference vs. control rats.

**Fig. 2.** The expression levels of CCK-1 receptor mRNA (A) and protein (B) in the pancreas. Figure shows representative blots from control and capsaicin-treated rats.
Effect of Capsaicin Treatment on Pancreatic Exocrine Secretion and Growth in Response to Exogenous CCK

Intravenous infusion of CCK-8 for 1 h caused a marked increase in protein secretion and a tendency to increase in fluid secretion in control rats, whereas it had no effects in capsaicin-treated rats (Fig. 4). Plasma CCK levels in capsaicin-treated rats at the end of CCK-8 infusion were not significantly different from those in control rats (Table 1).

Twice-daily intraperitoneal injection of 40 pmol/kg body wt CCK-8 for 7 days significantly increased pancreatic wet weight in both groups of rats compared with that in vehicle-treated control rats (Table 1). These results suggest that the vagal pathway is the primary site of action of CCK on the pancreatic secretion but not on pancreatic growth.

DISCUSSION

We found in the present study that pancreatic growth is promoted after chemical ablation of vagal afferent fibers in rats and that this growth is accompanied by increases in pancreatic protein, trypsinogen, and DNA content. Similar trophic effects on the pancreas have been demonstrated after repeated injections of exogenous CCK (30) or caerulein (8) and endogenous CCK stimulation by a high-protein diet (11) or trypsin inhibitor (28). In addition, several results also suggest that CCK plays an important role in the pancreatic growth after perivagal application of capsaicin. First, plasma CCK levels were higher in capsaicin-treated rats than those in control rats (Table 2). Second, intraduodenal infusion of trypsin prevented the increase in plasma CCK levels and pancreatic weight in capsaicin-treated rats (Table 1, Fig. 1). Third, the CCK-1 receptor antagonist loxiglumide prevented the pancreatic growth after chemical ablation of vagus afferent fibers (Fig. 1). Therefore, it is likely that increased endogenous CCK stimulates pancreatic growth after perivagal application of capsaicin and that elevated plasma CCK levels in capsaicin-treated rats is attributable to the decreased pancreatic protein, especially trypsin secretion into the duodenum.

The expression of pancreatic CCK-1 receptor mRNA and protein in the capsaicin-treated rats were not
significantly different from that in control rats (Fig. 2). However, previous studies demonstrated that pancreatic acinar cells possess both high- and low-affinity CCK-1 receptors (14, 34) and that the high-affinity receptors mediate CCK-stimulated enzyme secretion, whereas the low-affinity receptors mediate the inhibition of enzyme secretion observed at a high concentration of CCK (4, 35). Although CCK-1 receptor subtypes were not examined in the present study, the ablation of vagal afferent fibers might increase the ratio of high-affinity receptors to compensate for the decreased pancreatic exocrine secretion. Since the trophic effect of CCK is believed to be mediated by high-affinity CCK-1 receptors (6), an increased ratio of high-affinity receptors could be responsible for the pancreatic growth after chemical ablation of vagal afferent fibers.

We demonstrated in the present study that pancreatic growth is stimulated after chemical ablation of vagal afferent fibers by capsaicin and that CCK-1 receptor antagonist prevents the pancreatic growth. These results suggest that CCK exerts a trophic effect on the pancreas directly, not via capsaicin-sensitive vagal afferent fibers in rats. To confirm this hypothesis, we examined the effect of endogenous and exogenous CCK on pancreatic secretion and growth. As previously shown by Li and Owyang (20, 21), chemical ablation of vagal afferent fibers abolished the effect of exogenous and endogenous CCK on pancreatic secretion (Figs. 3 and 4). However, perivagal application of capsaicin showed no influence on pancreatic growth stimulated by endogenous and exogenous CCK (Table 1). These results further suggest that CCK exerts a trophic effect not via a vagal afferent pathway but directly on the pancreas in rats. In addition, previous studies demonstrated that CCK receptors present on the afferent neurons are lost after treatment with capsaicin (24, 25). Since loss of CCK receptors on the afferent nerves interrupts the effect of CCK via afferent fibers, these findings also support our view that CCK stimulates pancreatic growth and secretion by a different pathway in rats. However, the question of whether CCK stimulates pancreatic secretion by a capsaicin-sensitive vagal afferent pathway is not settled, and conflicting findings have also been reported (13, 19). Further studies seem to be needed to settle this issue.

Pancreatic fluid secretion was significantly increased after perivagal application of capsaicin (Figs. 3B and 4B). Although the precise mechanism of the hypersecretion remained unclear, we previously observed pancreatic fluid hypersecretion in the proliferative process of acinar cells after oral administration of the protease inhibitor camostat (44). Since the pancreatic growth is stimulated in the capsaicin-treated rats (Table 1), it is conceivable that acinar cell proliferation might influence pancreatic fluid hypersecretion in capsaicin-treated rats. On the other hand, intraduodenal administration of camostat, but not intravenous infusion of CCK-8, significantly stimulated pancreatic fluid secretion in control rats (Figs. 3B and 4B). The difference seems to arise from the effect of secretin (29) and different molecular forms of CCK released after camostat administration. A very recent study demonstrated that CCK-58, not CCK-8, is the only detectable endocrine form of CCK in rat (32).

In conclusion, we have demonstrated in the present study that pancreatic growth is promoted after chemical ablation of afferent vagal fibers by capsaicin and that CCK largely participates in the pancreatic growth. These results suggest that CCK exerts a trophic effect not via a vagal afferent pathway but directly on the pancreas in rats.

REFERENCES