Inhibitory Effect of CR-1409, a Competitive Inhibitor of Cholecystokinin, on Pancreatic Exocrine Secretion in the Conscious Rat

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The First Laboratory of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173, *the First Department of Internal Medicine, Tokyo Medical and Dental University Tokyo 113 and †the Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812

MIYASAKA, K., NAKAMURA, R., FUNAKOSHI, A. and KITANI, K. Inhibitory Effect of CR-1409, a Competitive Inhibitor of Cholecystokinin, on Pancreatic Exocrine Secretion in the Conscious Rat. Tohoku J. exp. Med., 1988, 155 (2), 165-172 — The inhibitory effect of CR-1409, a new glutaramic acid derivative and a competitive inhibitor for cholecystokinin (CCK), on the basal and CCK-stimulated pancreatic secretion was examined in the conscious rat in vivo. Rats were prepared with cannulae draining pure bile and pancreatic juice separately and with a duodenal cannula and right and left jugular vein cannulae. Plasma CCK level increased to 3.65±0.79 and 19.9±4.47 pM (mean±s.E.) by a 2-h infusions of 100 and 300 pmole/kg/hr of CCK-octapeptide (CCK-8), respectively. Simultaneous infusion of 170 nmole/kg/hr of CR-1409 completely abolished pancreatic responses to 100 pmole/kg/hr of CCK-8. Infusion of CR-1409 at rate of 57 nmole/kg/hr slightly but significantly inhibited CCK-8 (100 pmole/kg/hr)-stimulated secretion. Pancreatic responses to 300 pmole/kg/hr of CCK-8 were partially inhibited but not completely abolished by the 170 nmole/kg/hr of CR-1409. Neither the basal pancreatic secretion nor the bile secretion was affected by CR-1409. We conclude that CR-1409 inhibited CCK-stimulated pancreatic secretion in vivo. ——— CR-1409; pancreatic secretion; conscious rats

Several new glutaramic acid derivatives were found to be potent CCK-antagonists in the guinea-pig gallbladder (Makovec et al. 1985). In a recent in vitro study, CR-1409 (3, 4-dichloro-benzamido-N, N-dipentyl-glutaramic acid), one of these new glutaramic acid derivatives, was reported to selectively inhibit the amylase release from dispersed acini induced by cholecystokinin-octapeptide (CCK-8) (Niederau et al. 1986; Iwamoto et al. 1987). In the present study, the inhibitory effect of CR-1409 on CCK-stimulated pancreatic secretion was

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examined in the conscious rat in vivo. The present study was designed to establish the doses of CR-1409 needed to inhibit pancreatic secretion stimulated by submaximal (100 pmole/kg/hr) and supramaximal (300 pmole/kg/hr) doses of CCK (Nakamura et al. 1988).

**MATERIALS AND METHODS**

*Animal preparation*

Male Wistar rats (308-330 g) were obtained from Shizuoka Jikken Dobutsu (Shizuoka). Rats were fed commercial rat chow (CRF 1; Oriental, Tokyo) before surgery, during recovery and between experiments. Rats were prepared with cannulae draining pancreatic juice and bile separately and with a duodenal cannula. The operative procedures used in the study have been described in detail in previous publications (Miyasaka and Green 1984; Miyasaka et al. 1986). Briefly, after Enflurane anesthesia (Abbott, North Chicago, IL, USA) was delivered through a plastic face mask by means of a vaporizer, a midline abdominal incision was made. A cannula was inserted into the common bile duct proximal to the ampulla of Vater (0.025 in. inside diameter x 0.037 in. outside diameter; Silastic Medical Grade Tubing, Dow-Corning, Midland, MI, USA). Then, the common bile duct was ligated proximal to the pancreas near the liver, and the second cannula was inserted above the ligation below the bifurcation of the bile duct. An additional cannula was inserted into the duodenum to return bile-pancreatic juice (BPJ). Finally, cannulae were inserted into both the right and left jugular vein.

After the operation, rats were placed in modified Bollman-type restraint cages and were kept in free access to food and water, in a room with filtered air at 24°C, and light was scheduled from 05:00 through 17:00. Experiments were conducted after 5-hr of fasting between the third and sixth postoperative days. During recovery and between experiments, BPJ was continuously returned to the intestine.

*Experimental design*

BPJ was collected every 30 min period. The sample collected during the first 30-min period during which the previously pooled BPJ was infused, was not used for assay. The volume of pancreatic juice was measured by a Hamilton syringe and 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 pancreatic juice, which was then returned to the intestine by a syringe pump (Harvard Apparatus compact infusion pump, Harvard Apparatus, Southnatick, MA, USA) for the next 30-min collection. After the 90-min basal collection, CCK-8 (100 pmole/kg/hr or 300 pmole per kg/hr) was infused at a rate of 1 ml/hr for 2 hr. Fifty seven or 170 nmole/kg/hr of CR-1409 was simultaneously infused with 100 pmole/kg/hr of CCK-8. In another group of rats 170 nmole/kg/hr of CR-1409 was infused with 300 pmole/kg/hr of CCK-8. The effect of the infusion of CR-1409 on basal pancreatic secretion was also examined. CCK-8 and CR-1409 were dissolved in 1% BSA-saline solution.

At the end of each experiment (after 2 hr CCK infusion without CR-1409) a 5 to 6 ml blood sample was drawn from jugular vein cannula by a heparinized syringe. To examine basal CCK concentration, rats were sacrificed before the CCK infusion and blood was obtained. Blood samples were centrifuged at 3000 rpm, for 15 min at 4°C. Plasma samples thus obtained were kept at −70°C until the CCK bioassay.

*Assays*

Bicarbonate concentration was measured immediately after each collection of pancreatic juice by a Natelson microgasometer using a 10 μl sample (Natelson 1951; Natelson and Menning 1955). 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 (Keller et al. 1958).

Plasma CCK concentrations were measured by a bioassay using dispersed acini as described by Liddle and Louie (Liddle et al. 1984; Louie et al. 1986). Isolated rat pancreatic acini were prepared by collagenase digestion of pancreases from fasted, ovariec-tomized female Sparague-Dawley rats as previously described (Liddle et al. 1984). For the measurement of plasma CCK levels in rats given CCK, 1 to 2 ml plasma samples were obtained. For the measurement of CCK level in rats at basal state, a 3 ml plasma sample was obtained from each rat, two 3 ml samples were combined into one 6 ml sample and used for the CCK measurement. CCK was extracted from these plasma samples by absorption onto Sep-Pak cartridges, washed with 20 ml water, and eluted with 1 ml of acetonitrile: water (1:1, vol./vol.) into a polyethylene scintillation vial and dried with a flow of nitrogen at 45°C (Louie et al. 1986). One ml aliquots of acini suspension were added to the vial containing the plasma extracts or various concentrations of CCK-8 and incubated for 30 min at 37°C. Amylase into the medium and total acinar amylase contents were measured by using the blue starch polymer (Neo-Amylase test; Daiichi Pure Chemicals Co., Ltd., Tokyo) as the substrate. Values were compared with values of a standard curve of CCK-8 and results were expressed as CCK-8 equivalents.

**Drugs**

CR-1409 (sodium salt) was a generous gift from Prof. L.A. Rovati and Prof. I. Setniker of the Rotta Research Laboratorium, Milano, Italy. Synthetic CCK-octapeptide sulfate was purchased from Peptide Institute, Inc. (Osaka). Bovine serum albumin (BSA); soybean

![Graph](image.png)

**Fig. 1.** Pancreatic responses to 100 pmole/kg/hr of CCK-8. This dose of CCK-8 significantly increased fluid, bicarbonate and protein outputs. Values are means ± s.e., n = 5. *p < 0.05 against respective basal values.
tyrosin inhibitor (type I-S); chromatographically purified collagenase (type IV); were from Sigma Chemical (St. Louis, MO, USA), Minimal Eagle’s medium amino acid supplement from GIBCO Laboratories, Life Technologies, Inc. (Grand Island, NY, USA), and HEPES from Calbiochem-Behring (La Jolla, CA, USA).

Statistical analysis

Experimental values were expressed in terms of mean ± s.e. These values were analyzed by Student’s t-test or one-way analysis of variance (ANOVA), followed by the Newman-Keul’s multiple comparison test. Differences were considered to be significant at p < 0.05.

RESULTS

Effects of CR-1409 on pancreatic secretion stimulated by 100 pmole/kg/hr of CCK-8

The infusion of 100 pmole/kg/hr of CCK-8 significantly and continuously increased fluid, bicarbonate and protein outputs (Fig. 1). Plasma CCK level
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increased to 3.65±0.79 pM (n=4) after the 2-hr CCK-8 infusion from the basal level of 0.46±0.05 pM (n=9).

CR-1409 (170 nmole/kg/hr) prevented the pancreatic response to CCK-8 (Fig. 2). None of fluid, bicarbonate and protein outputs was increased by the infusion of CCK-8 under the simultaneous infusion of CR-1409. CR-1409 (57 nmole/kg per hr) partially inhibited pancreatic responses to CCK-8. Changes in fluid and bicarbonate outputs were not significant in comparison to basal levels, as shown in Fig. 2.

The increments of protein output during CCK-8 infusion with or without

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<th>TABLE 1. Increments of protein output above basal levels during 100 pmole/kg/hr CCK-8 infusion with or without CR-1409</th>
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<tr>
<td>CCK-8, 100 pmole/kg/hr*</td>
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<td>CCK-8 with 57 nmole/kg/hr of CR-1409</td>
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<td>CCK-8 with 170 nmole/kg/hr of CR-1409</td>
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Values are calculated as follows: an average value per 1 hr during 2-hr infusion of CCK-8 and/or CR-1409 minus the value during 1 hr before the infusion. F (2,13) = 27.68. The inhibitory effect of CR-1409 on incremental protein outputs induced by CCK-8 is significant by ANOVA and + indicates a significant difference by Newman Keul's multiple comparison test.

Fig. 3. Pancreatic responses to 300 pmole/kg/hr of CCK-8. This dose of CCK-8 significantly increased fluid, bicarbonate and protein outputs (n=6).
CR-1409 were shown in Table 1. CR-1409 inhibited the increments of protein secretion stimulated by CCK-8.

**Effects of CR-1409 on pancreatic secretion stimulated by 300 pmole/kg/hr of CCK-8**

The infusion of 300 pmole/kg/hr of CCK-8 continuously increased fluid bicarbonate outputs as observed in the stimulation of 100 pmole/kg/hr of CCK-8, however, protein output peaked during 30-60 min collection period and then slightly decreased (Fig. 3). Plasma CCK level increased to 19.9±4.47 pM (n=5).

CR-1409 (170 nmole/kg/hr) significantly inhibited pancreatic secretion stimulated by 300 pmole/kg/hr, although it could not completely abolish the increase in protein and fluid outputs responding to CCK-8. Bicarbonate response was completely inhibited (Fig. 4).

**Effects of CR-1409 on basal pancreatic secretion**

The infusion of 170 nmole/kg/hr of CR-1409 did not affect basal secretion in terms of fluid, bicarbonate and protein outputs, and the secretion of bile was also not affected by CR-1409 (data are not shown).

**DISCUSSION**

The present study demonstrated that CR-1409 inhibits CCK-8 stimulated pancreatic secretion in vivo. Continuous infusion of 100 pmole/kg/hr of CCK produced an increase in plasma CCK level to 3.76 pM from the basal level of 0.45 pM, which is considered to be within the physiological range (Liddle et al. 1984; Louie et al. 1986). Liddle et al. (1984) have shown that plasma CCK level increased to 6-10 pM after the feeding of a balanced liquid meal or trypsin.
inhibitor. However, this large increase was only transient in the period between 7.5-15 min after feeding. Then, the plasma CCK level decreased to 1.5 pM and remained at that level for 30-120 min. On the other hand, 300 pmole per kg/hr, a three times higher infusion rate is considered to have induced a supramaximal plasma concentration and produced the transient increase of protein output (Fig. 3). It appears therefore, that a continuous infusion of 100 pmole/kg/hr of CCK is a submaximal stimulating dose in rats.

The inhibitory effect of CR-1409 was dose dependent. A smaller dose of CR-1409 (57 nmole/kg/hr) partially inhibited the protein output increase induced by 100 pmole/kg/hr of CCK-8 but could not abolish it totally. A three times larger dose (170 nmole/kg/hr) of CR-1409 completely abolished the pancreatic response to 100 pmole/kg/hr of CCK. However, the larger dose of CR-1409 (170 nmole/kg/hr) was not sufficient to induce a complete inhibition of pancreatic secretory response, when a larger dose of CCK-8 (300 pmole per kg/hr) was administered. Indeed, a slight but significant increase in protein output by CCK-8 was still preserved. Thus, it was observed that CR-1409 inhibited CCK-stimulated pancreatic secretion in vivo.

Proglumide, another glutaramic acid derivative, that was developed in 1967 (Rovati et al. 1967) has been known to have a CCK-antagonistic effect (Hahne et al. 1981; Jensen et al. 1983). However, we have recently reported that proglumide markedly increased the bile secretion and stimulated the basal pancreatic secretion via a mechanism independent of CCK receptor (Miyasaka et al. 1987). In contrast, CR-1409 did not affect either basal pancreatic secretion or bile secretion. Therefore, it is suggested that CR-1409 may be a useful agent to determine the involvement of CCK release, in vivo, and the minimal and enough dose is about 170 nmole/kg/hr.

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References


