Effects of intraduodenal administration of a low dose of cholecystokinin (CCK) antagonist (CR-1505) on plasma CCK concentration, intestinal CCK content, and levels of CCK mRNA

NAOKO SAZAKI,1 KYOKO MIYASAKA,1∗ MASAHIRO MATSUMOTO,2 and AKIHIRO FUNAKOSHI2

1Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo, 173 Japan
2National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku Fukuoka, 815 Japan

Abstract: The effects of the intraduodenal administration of a low dose of CR-1505 for 3–7 days on the gene expression of cholecystokinin (CCK), plasma CCK concentration, and CCK content in the intestinal mucosa were examined in rats. The simultaneous changes of protein and enzyme content in the pancreas were also determined. CR-1505 was infused continuously into the duodenum at a dose of 3 mg/kg per day, calculated to correspond to a dose of 150–200 mg/day in humans. Seven days after the administration of CR-1505, a liquid meal (4.5 kcal/3 ml) was introduced into the stomach and changes in the intestinal CCK content and plasma CCK concentration were examined. The level of CCK mRNA in the intestine was significantly higher in rats treated with CR-1505 than in control rats. The plasma CCK concentration, the CCK content of the intestinal mucosa, and the composition of pancreatic enzymes did not significantly differ in rats treated with CR-1505 than in control rats. The plasma CCK concentration, the CCK content of the intestinal mucosa, and the composition of pancreatic enzymes did not significantly differ in rats treated with CR-1505 and the untreated controls. In control rats, the administration of the liquid meal increased the plasma CCK concentration and significantly decreased the intestinal CCK content in water extracts, but did not affect the amount extracts in acid whereas the ingestion of the meal did not cause any significant changes in rats treated with CR-1505. These findings indicate that a low dose of CR-1505 stimulates the gene expression of CCK without enhancing CCK release or exerting an effect on the pancreas.

Key words: CCK, gene expression, CCK antagonist

Introduction

Cholecystokinin (CCK) is physiologically important for stimulating pancreatic enzyme secretion and inducing gallbladder contraction,1 and it has a trophic effect on pancreatic acini.2,3 Many kinds of CCK receptor antagonists have been developed,4 including CR-1505 (loxiglumide), which has a highly selective and specific effect.5 These CCK antagonists inhibit the trophic effect of CCK when administered with CCK,6–8 and cause hypotrophy of the pancreas when administered alone without CCK.9 Thus, endogenously-released CCK is thought to be necessary for the maintenance of the normal exocrine function of the pancreas, as well as for stimulating enzyme secretion. However, the regulatory mechanism responsible for the synthesis and release of CCK has not been fully clarified. The main source of circulating CCK is the endocrine cells in the proximal intestinal mucosa, and the tissue content of CCK is considered to represent a balance between CCK synthesis, storage, and secretion. Previously, Schmidt et al.10 and Funakoshi et al.11 have reported that the intravenous infusion of a CCK antagonist in humans increased the plasma level of CCK-like-immunoreactivity, although the bioactivity of CCK was decreased. Enhanced CCK release may result in an increase in CCK synthesis (increased CCK mRNA levels) to restore the pool. More recently, we reported12 that the intragastric administration of 60–300 mg/kg per day of CR-1505 for 3 days increased the plasma CCK concentration and the level of CCK mRNA in rats. However these doses were extraordinarily high. Therefore, in the present study, we used a low dose of CR-1505 (3 mg/kg per day). This dose was calculated to correspond to a dose of 150–200 mg/day in humans, and is considered to be sufficient to prevent the biological activity of physiologically released endogenous CCK.13,14 We examined the effects of this dose on the level of CCK mRNA in

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the intestine, plasma CCK concentration, and intestinal CCK content, and compared the changes of protein and enzyme content in the pancreas in untreated controls and animals treated with CR-1505.

Materials and methods

Animals

We used 77 young male Sprague-Dawley rats (5-6 weeks old, 147-173 g body weight). They were obtained from Shizuoka Jikken Dobutsu (Shizuoka, Japan), and were fed commercial rat chow (CRF-1; Oriental Yeast Co., Tokyo, Japan). Eleven and 23 rats, respectively, were assigned for 3- and 7-day treatment with 3 mg/kg per day of CR-1505, and 9 and 19 rats were treated with distilled water as controls. These rats, except for 18 rats receiving the 7-day treatment with or without CR-1505 (9 in each group), which were sacrificed 7 min after the ingestion of a liquid meal, were sacrificed to examine plasma and tissue CCK concentrations, pancreatic composition and luminal trypsin activity. To examine the level of CCK mRNA, 15 rats were divided into five groups (3 in each group): 3-day treatment with 3 mg/kg per day or with 15 mg/kg per day of CR-1505; 7-day treatment with 3 mg/kg per day of CR-1505; and 3- or 7-day treatment with distilled water.

Animal preparations

The animals were anesthetized with an intraperitoneal injection of 40 mg/kg body weight of pentobarbital sodium (Abbott Laboratories, North Chicago, Ill.), and a midline abdominal incision was made. A cannula (polyethylene tubing, Clay Adams, N.J.: 0.762 mm internal diameter, 1.219 mm internal diameter) was inserted into the duodenum near the ampulla of Vater. The cannula was connected to an osmotic pump (2ML1; Alza Corp., Palo Alto, Calif.) filled with 20 mg/2 ml of CR-1505 (a generous gift from Kaken Pharmaceutical Co., Tokyo, Japan) or distilled water, and 3 mg/rat per day of CR-1505 or water was infused continuously into the intestinal lumen. The osmotic pump was placed in the right abdominal cavity. The rats were maintained in laboratory cages (one to four rats per cage), and had free access to food and water.

Rats were anesthetized with ether 3 or 7 days after the operation without previous fasting. Then 3-7 ml of blood was withdrawn from the abdominal aorta into a heparinized syringe, and the pancreas and the proximal quarter of the small intestine were removed. The blood samples were placed in ice-chilled tubes containing ethylene diamine tetraacetic acid (EDTA) and promptly centrifuged at 3000 rpm for 15 min at 4°C; the plasma samples were stored at -70°C until use. The pancreas was weighed and frozen in liquid nitrogen for the measurement of enzyme and protein content. The proximal small intestine was washed with 20 ml of cold distilled water, then opened longitudinally. The intestinal mucosa, removed by gently scraping the surface with a spatula, was promptly frozen in liquid nitrogen. The tissues were then lyophilized for subsequent assays. The intestinal contents were also frozen and lyophilized for the measurement of trypsin activity.

The levels of CCK mRNA were measured after the administration of 3 and 15 mg/kg per day of CR-1505 for 3 days to examine the dose-dependent effect. To examine the time dependency, the levels were measured after 3- or 7-day treatments with 3 mg/kg per day of CR-1505. The intestinal mucosa was removed an promptly frozen without being weighed (n = 3 for each group).

For examination of the effect of food stimulation on day 7 of the experiment, 4.5 kcal/3 ml of a liquid meal (principal ingredient, casein; Clinimeal; Eisai Co., Ltd., Tokyo, Japan) was administered through an orogastric tube and the rats were sacrificed 7 min later.

Assays

Slot-Blot analysis. Expression of specific mRNA was measured by slot-blot analysis, using the method of Kanayama and Liddle,15 with slight modification. Briefly, 10 µg of total RNA was immobilized on a nylon membrane (Hybond-N; Amersham Japan, Tokyo), using a slot-blot apparatus (Biodot SF; Bio-Rad Japan, Tokyo). Hybridization was carried out with the reverse transcriptase-polymerase chain reaction (RT-PCR) product of 343 bp cDNA, which was prepared from RNA of rat intestine, using the sense primer 5'-AGCCGGTAGTCCCTGTAGAA-Y and anti-sense primer 5'-GTGCGTGGTTTCTCAT-3' for CCK16 labelled with [32P] dCTP. Hybridization with a β-actin probe (443 bp; Wako Pure Chemical Co., Osaka, Japan) was carried out as a control. The membranes were exposed to X-ray film (X-Omat, XAR-5; Kodak Inc., Rochester, N.Y.). Autoradiograms were analyzed with a computer scanner (Sharp JX-32F3: Sharp Electric Co., Osaka, Japan) and an NIH image program operated with a Macintosh computer (Apple Computer Inc., Cupertino, Calif.). The values were expressed as percent changes of CCK mRNA/β-actin mRNA. Values for β-actin mRNA were constant.

RNA samples were separated by electrophoresis in 1% agarose gel, immobilized on a nylon membrane, and examined qualitatively by the Northern transfer method. Filters were hybridized with the CCK and β-