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Cholecystokinin is not a major regulator in the digestive system in the chicken

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Abstract. To find out whether physiological concentrations of cholecystokinin (CCK), a gastrointestinal hormone in mammals, are also active in chickens, the pancreatic amylase secretory response to CCK-8 was investigated in vitro. Rat pancreatic acini responded to the physiological concentration of CCK-8, but in chickens amylase release was induced at a concentration of CCK-8 1000 times higher than that observed in rats. In another experiment, biliary flow was tested with several doses of CCK-8. The bile flow was stimulated in a dose-dependent fashion, but a significant enhancement was not obtained at a concentration of 0.5 μg CCK-8/kg body weight, which was considerably higher than physiological ones. It is concluded that endogeneous CCK does not have an important role in the digestive system in the chicken.
Key words. Cholecystokinin; exocrine pancreatic secretion; bile flow; chicken; rat.

Mammalian gastrin and cholecystokinin (CCK) share a common biologically active C-terminal pentapeptide sequence and have a similar range of biological activities. This has led to the suggestion that these peptides have a common evolutionary history and have arisen from a common ancestral gene[1]. In birds and mammals, however, the amino acid sequences of gastrin and CCK have evolved in different ways, and the structure of chicken gastrin is similar to mammalian CCK rather than to mammalian gastrin[2].

In mammals, the major roles of CCK are known to be stimulation of pancreatic exocrine secretion and gallbladder contraction, and inhibition of gastric emptying. In rats, postprandial plasma CCK concentrations are sufficient to stimulate pancreatic enzyme secretion[3]. CCK also has a stimulatory effect on exocrine secretion in turkeys[4] and in chickens[5]. However, the responses were obtained by pharmacological dose of CCK. Furthermore, in other studies, the administration of pharmacological concentrations of CCK failed to stimulate juice flow and protein output from the pancreas in chickens and ducks[6], and at physiological doses, CCK does not stimulate exocrine secretion in laying hens[7]. Thus, the role of CCK in stimulating avian pancreatic secretion remains to be established. The present study compared the direct effect of CCK-8 on the isolated pancreatic acini of chicken with its effect on those of the rat; the bile secretion response to CCK-8 was also tested.

Methods

Animals. Day-old Single Comb White Leghorn male chicks, purchased from a local supplier (Hattori Hatchery Co., Ltd., Nagoya, Japan), were maintained in individual cages in a temperature-controlled room (30 °C), with continuous lighting. They were given ad libitum a commercial chick starter (Marubeni Shiryo Ltd., Tokyo, Japan). Wistar rats purchased from a local supplier (Japan SLC, Inc., Hamamatsu, Japan) were housed in a temperature-controlled room (25 °C) under light-control (12D:12L), and were given a commercial rat chow (Nihon Nousan Kougyou Co., Ltd., Yokohama, Japan) and water ad libitum.

Dispersed pancreatic acini study. Isolated pancreatic acini were prepared according to a modification of the procedure published previously[8]. Briefly, chickens and rats weighing about 200 g and 300 g, respectively, were fasted overnight with free access to water. The animals were killed by decapitation and the pancreas quickly removed. The pancreatic parenchyma were injected with incubation medium containing 200 U/ml collagenase (Type VII, Sigma Chemical Co., St. Louis, MO, USA) on a warm (37 °C) laboratory dish. The incubation medium contained 12.5 mM-Hepes, 124 mM-NaCl, 4.8 mM-KCl, 1.2 mM-KH_2PO_4, 1.2 mM-$MgSO_4$, 5.0 mM-$NaHCO_3$, 2.0 mM-$CaCl_2$, 3.1 mM-sodium fumarate, 49.8 mM-sodium pyruvate, 6.8 mM-glutamic acid, 11.1 mM-glucose, 0.05% (wt/vol)-SBTI (Type II-S, Sigma Chemical Co., St. Louis, MO, USA) and 0.2% (wt/vol)-BSA (Fraction V, Sigma Chemical Co., St. Louis, MO USA). The incubation medium was adjusted to pH 7.4 and saturated with 100% O_2 before and during the incubation. The pancreas was transferred to a polyethylene flask and incubated in a shaking water bath at 37 °C for 20 min. The medium was replaced with fresh collagenase solution, and the pancreas then chopped with scissors into small pieces and incubated for another 20 min. Thereafter, the tissue was dissociated gently, using polyethylene pipettes of decreasing diameter, and filtered through nylon mesh of 175 μm. Acini were purified by sedimentation through albumin gradient (40 mg BSA/ml) and resuspended in the incubation medium. They were incubated with various concentrations of CCK-8 (Peptide Institute Inc., Osaka,

Japan) for 30 min. The CCK-8 concentrations used in this study were 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} M for rat and 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M for chicken. Amylase concentration was determined by the blue starch method[9]. Amylase increment was expressed as a relative value of the total amount of amylase in an aliquot.

Bile secretion study. Eight chickens aged 4–5 weeks weighing 256 ± 6 g were fasted overnight but were allowed free access to water. Birds were anesthetized with urethane (1.5 g/kg b. wt) by multiple i.p. injections in the abdomen, then placed in a heated room maintained at body temperature (37 °C). The left ventral side of the abdominal cavity was opened and the isthmus (the gizzard-proventriculus junction) was ligated to prevent the entry of gastric acid into the duodenum. Then the right ventral side of the abdominal cavity was opened to expose the gall bladder and the duodenum. The cystic duct was cannulated with polyethylene tubing (i.d. 1 mm) and the hepaticoenteric duct was ligated. The bile flow was measured at 10 min intervals. The basal rate of flow was established at least 40 min prior to the administration of CCK-8, which was given by rapid i.v. injections in random order to the same bird. The CCK-8 concentrations used in this study were 0.5, 5 and 15 µg/kg b.wt. Results were expressed as peak percentage of basal rates, using the values at 10 min before each infusion as basal.

Statistical analysis. One way analysis of variance was applied to test the response of amylase secretion to the various concentrations of CCK-8 in the dispersed acini from the same species. Statistical significance of bile secretory responses to CCK-8 was analyzed using a paired t-test. All of the data analysis was done using a commercially available statistical package[10].

Results

Dose–response effects of CCK-8 on amylase release from dispersed pancreatic acini of chicken and rat are shown in figure 1. In each species, amylase release was enhanced by CCK-8 in a dose-dependent manner. However, the efficiency and potencies of CCK-8 differed approximately 1000-fold between the two species. Maximal amylase release was observed at a concentration of 10^{-11} M in rat and 10^{-8} M in chick, and the ED_{50} was at 10^{-12} M and at 10^{-9} M, respectively.

Dose–response effects of CCK-8 on the bile flow in chicken are shown in figure 2. Biliary flow was enhanced by increasing levels of exogenous injected CCK-8. The maximal response at 15 µg CCK-8/kg reached 200% of the basal secretion, but no significant increase was obtained at 0.5 µg CCK-8/kg b.wt.

Discussion

The potency and efficacy of CCK-8 in stimulating pancreatic secretion in the rat were similar to those reported by others[11,12]. The dose–response curve of CCK-8 in chicken demonstrated a direct effect of CCK-8 on chicken acinar cells for the first time. Plasma CCK-8 concentrations in chickens have been reported in several studies[13–15]. The concentration varies from 7 pM in the basal state to 20 pM in the postprandial state. Because the effective dose observed in the present study is quite high (response appeared at 1 nM), it seems unlikely that chicken exocrine pancreatic secretion is stimulated by endogenous CCK-8 alone.

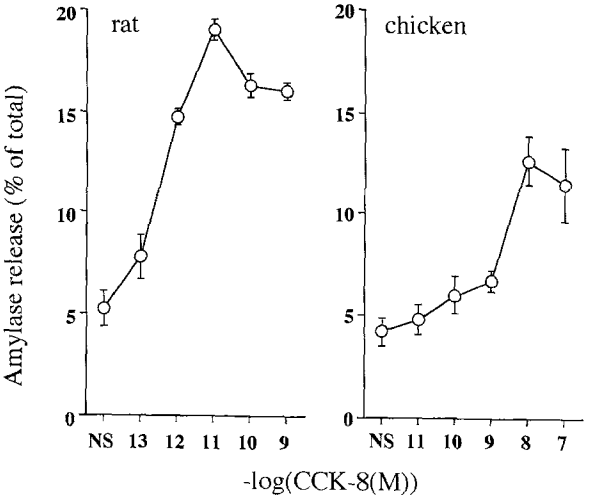

Figure 1. Dose–response effects of CCK-8 on amylase release from isolated pancreatic acini of rat (left panel) and chicken (right panel). Acini were incubated with various concentrations of CCK-8 for 30 min. Amylase release was expressed as the percentage of the total amylase present in an aliquot. Each point represents mean ±SE of five replicates.

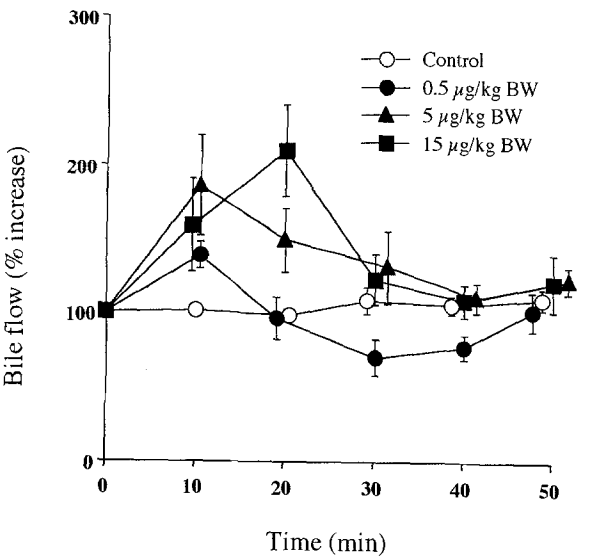

Figure 2. Dose–response effects of CCK-8 on time course of biliary secretion from anesthetized chickens. Various concentrations of CCK-8 were administered at time 0 by rapid i.v. injection in random order to the same bird. Each point represents mean ±SE of the percent increase of the basal secretion, the value at 10 min before each injection, from eight replicates.

Continuous infusion of CCK evoked biliary secretion but not pancreatic secretion in conscious laying hens[7]. In the present study, bile flow was stimulated by CCK-8, but the increase was not significant with the lowest dose. The dose used in this study was also very high compared to plasma concentration of CCK-8. The threshold dose of CCK-8 to stimulate gall-bladder contraction was 100 pM in the chicken[6]. Thus the present results are consistent with the theory that to enhance the bile flow, a CCK concentration higher than 100 pM is required. According to Vigna et al.[17], the membrane from chicken pancreas did bind to ^{125}I-labeled CCK, but binding was much lower than that to mouse membranes. The small number of receptors may also be associated with the weak response to CCK in the chicken. The present study suggests that endogenous CCK alone is not sufficient to account for postprandial increases in bile flow and exocrine pancreatic secretion.

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