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The action of the cholecystokinin-A receptor antagonist, devazepide, on the digestive system of the chicken

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Abstract. The influence of the cholecystokinin (CCK)-A receptor antagonist, devazepide (DVZ), on the chicken digestive tract was investigated. The passage of food from the crops of birds treated with DVZ was not significantly different from that of the control. DVZ treatment did not inhibit the biliary flow stimulated by the CCK analogue, caerulein. Dispersed chicken pancreatic acini stimulated with CCK were treated with various concentrations of DVZ. At 10^{-5} M, DVZ completely inhibited amylase release; this concentration was much higher than those reported to have similar effects in mammals. The results suggest that the action DVZ as a CCK antagonist in the chicken is very weak.

Key words. Devazepide; cholecystokinin; caerulein; amylase secretion; chicken.

Cholecystokinin (CCK) is recognized as the principal hormone involved in the stimulation of pancreatic exocrine secreation, stimulation of gall bladder contraction and inhibition of gastric emptying and food intake. Good progress has recently been made in the development of specific antagonists for CCK receptors. The benzodiazepine, devazepide (DVZ), is an antagonist of CCK-A receptors in mammals. DVZ inhibits the action of CCK on gastric emptying, pancreatic secretion, gall bladder contraction and food intake[1–4]. However, these results using DVZ are confined to mammalian species, and previous reports of the action of DVZ in avian species have been inconsistent. DVZ failed to inhibit prancreatic flow stimulated by CCK[5] and to improve food intake[6, 7], but Covasa and Forbes[8] reported that CCK plays a role as feeding regulator since intraperitoneal (i.p.) administration of DVZ increased food intake in broiler chickens. Richardson et al.[9] found that the reduced food intake of white crowned-sparrows induced by CCK was attenuated by DVZ but not by the CCK-B receptor antagonist L365,260, whereas DVZ alone did not increase ingestion of 20% sucrose solution. However, these findings did not test the ability of DVZ to bind to CCK-A receptors, and it is not possible to conclude that endogenous CCK acts as a satiety factor in the chicken. In the present study, therefore, we have systematically investigated the role of DVZ on the chicken digestive tract with special reference to food passage, bile flow and pancreatic amylase secretion.

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Material and methods

Animals and treatments

Day-old Single Comb White Leghorn male chicks were purchased from a local supplier (Hattori Hatchery Co. Ltd., Nagoya, Japan) and were given a commercial chick starter mash (Marubeni Shiryo Ltd., Tokyo, Japan) in all experiments. All birds were maintained in individual cages, and exposed to continuous lighting in a temperature-controlled (28 °C) room. DVZ was a gift from Merck Sharp & Dohme Research Laboratories (Hollow, Essex, England).

Crop emptying study. The chickens (28–29 day-old, 270–340 g) fasted overnight with free access to water, and were distributed into eight groups of six birds each so that mean body weights were as uniform as possible. The birds then received a single meal of the experimental diet. The diet was mixed with water at the ratio of 2:3 (wt:wt) and birds were tube-fed 5 ml of slurry through the esophagus into the crop. In the present study, a semi-purified diet was used and its composition was as follows (g/kg): soybean protein 226, L-methionine 2.9, L-threonine 1.2, glycine 4.2, maize oil 55, maize starch 547.7, cellulose 100, mineral mixture 58.5, vitamin mixture 2, choline chloride 1.5, and inositol 1. The compositions of mineral and vitamin mixtures were described by Furuse and Okumura[10]. The doses of DVZ were 0, 100, 500 and 1000 μg/kg body weight. DVZ was injected i.p. 30 min before the food intubation. The skin over the crop was incised and clamps were attached across the upper and lower crop junctions under light anesthesia with diethyl ether, 1 or 2 h after the administration of meal. The crop was cut distal to the clamps, and crop content was removed and dried at

55 °C for 24 h and weighed. The crop emptying rate was assessed by measuring the dry weight of the crop content and expressed the relative weight of the crop content to the amount of food intubated.

Bile flow study. Following an overnight fast eight chickens (289–330 g) aged 28–30 days were anesthetized with 25% solution of urethane (1.5 g/kg body weight) administered by multiple i.p. injections into the abdomen. Each bird was then placed in a heated room maintained at body temperature (37 °C). The abdominal cavity was opened and the isthmus (the gizzard-proventriculus junction) was ligated to prevent acid flow into 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 caerulein. DVZ (1 mg/kg boy weight) or vehicle was given by i.v. injection in random order 10 min before caerulein treatment. DVZ was dissolved in 0.25% methylcellulose. Caerulein was administered to chickens at 4 µg/kg body weight. Caerulein (Ceosunin®) was donated by Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan.

Amylase secretion study. Isolated chicken acini were prepared by the modified method of Bruzzone et al.[11] A 21-day-old chicken (220 g) was killed by decapitation. The pancreas was removed and 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 nM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 5.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 3.1 mM sodium furmarate, 49.8 mM sodium pyruvate, 6.8 mM glutamic acid and 11.1 mM glucose, 0.05%-SBTI type II-S (Sigma Chemical Co., St. Louis, MO, USA) and 0.2% BSA (Fraction V, Sigma Chemical Co., St. Louis, MO, USA); pH was adjusted to 7.4 and the medium was gassed 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. After replacement with fresh collagenase solution the pancreas was chopped into small pieces with scissors and incubated for another 20 min. Thereafter, tissue was moderately dissociated using decreasing diameter polyethylene pipettes and filtered through nylon mesh of 175 µm. Acini were purified by sedimentation through an albumin gradient (40 mg BSA/ml), resuspended in the incubation medium and incubated with various concentrations of stimulant or/and antagonist for 30 min. Amylase concentration was determined by the blue starch method[12]. Amlyase release was expressed as a relative value of total amount of amylase in an aliquot. In a previous report[13], a CCK dose response curve was obtained in the range of 10^{-11} to 10^{-7} M. The maximal secretion was obtained at concentration of 10^{-8} M and this concentration was applied in the present study. The antagonistic effect of DVZ for miximal pancreatic amylase secretion was determined from 10^{-9} to 10^{-5} M DVZ.

Statistical analysis. Two-way analysis of variance was applied to the results of food passage from the crop. Statistical significance in bile secretory responses was analyzed by analysis of variance considering the chick as main plots and time after treatments as subplots. The effect of DVZ on amylase release from the dispersed

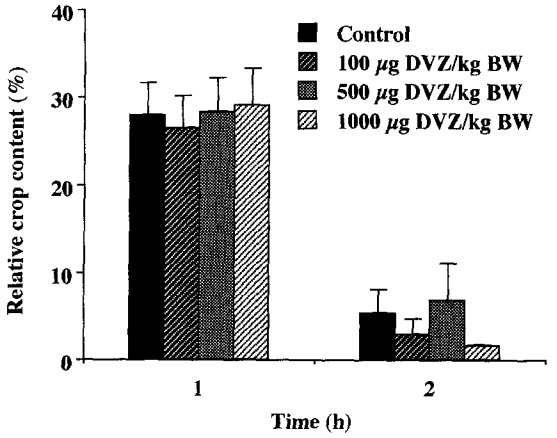

Figure 1. Crop-emptying rate of chickens treated with various concentrations (0, 100, 500 and 1000 µg/kg body weight) of CCK-A receptor antagonist, devazepide (DVZ). The diet was given as a single meal of about 2 g through the stomach tube. Values are expressed as weight of the meal remaining in the crop 1 and 2 h relative to the amount initially infused. DVZ was i.p. injected 30 min before food intubation. Values are means ± SE of six birds.

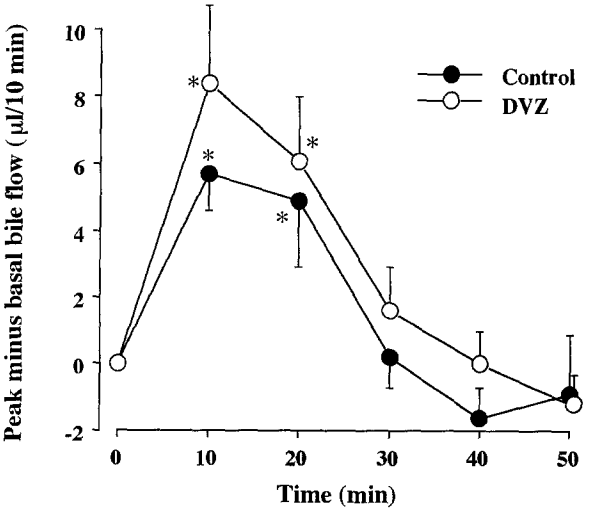

Figure 2. Effects of CCK-A receptor antagonist, devazepide (DVZ), on bile flow stimulated with caerulein. Chickens were intravenously injected with caerulein in 4 µg/kg body weight. DVZ (1 mg/kg body weight) or its vehicle (0.25% methly cellulose) was injected i.v. 10 min before caerulein treatment. Values are means ± SE of eight birds. *Significantly different for the initial value at $p < 0.05$.

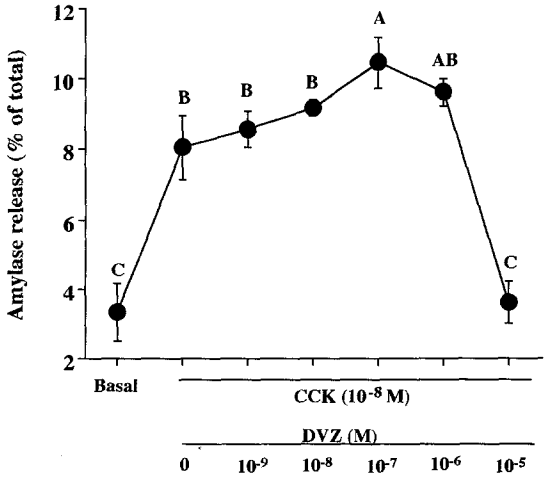

Figure 3. The response to several concentrations of CCK-A receptor antagonist, devazepide (DVZ), on amylase release from dispersed pancreatic acini stimulated by CCK. Values are means ± SE or five observations. Values with different letters differ significantly at $p < 0.05$.

acini was determined for one-way analysis of variance and followed by Duncan's multiple range test. The data analysis was done using a commercially available statistical package[14].

Results

Figure 1 shows crop emptying of food in chickens treated with various doses of DVZ. No significant effects were observed in DVZ, $F(3, 35) = 1.05$, $p = 0.382$, or in interaction between DVZ × time, $F(3, 35) = 0.32$, $p = 0.814$. Only the effect of time was significant ($F(1, 35) = 105.36$, $p\ 0.001$), less food being retained 2 h after intubation.

The effect of DVZ on bile flow stimulated by caerulein is shown in figure 2. Bile flow was enhanced by caerulein treatment ($F(5, 77) = 13.50$, $p = 0.0001$), but there were no significant differences between vehicle and DVZ treatment ($F(1, 14) = 0.97$, $p = 0.342$). Figure 3 indicates the influence of several concentrations of DVZ on amylase release from dispersed pancreatic acini stimulated by CCK. Pancreatic amylase secretion was not inhibited by 10^{-6} M of DVZ, but at the highest level DVZ (10^{-5} M) it was inhibited ($F(6, 28) = 14.27$, $p = 0.001$).

Discussion

CCK and gastrin are considered to have a common evolutionary history and to have arisen from a common ancestral gene[15]. Mammalian gastrin and CCK share a common biologically active C-terminal pentapeptide sequence and have a similar spectrum of biological activities. The structure of chicken gastrin resembles mammalian CCK rather than mammalian gastrin, but mammalian and avian gastrin have similar biological properties[16]. There is evidence that the agonist potencies of CCK receptor in birds resemble those in mammals, but amylase release from dispersed pancreatic acini following stimulation by CCK[13] and caerulein[17] is very weak. It seems, therefore, that there may be differences in antagonist potencies.

Campbell et al.[5] suggested that DVZ may show some species-specificity for the CCK-A type receptor, because it failed to inhibit pancreatic flow in anesthetized turkeys stimulated by CCK at 1 μmol/kg body weight. This suggestion is reasonable, since maximally stimulated amylase release by CCK was completely inhibited by DVZ at 30 nM in rats[18] but at 10 μM in the present study. Furthermore, the effect of DVZ on chicken pancreatic amylase secretion stimulated by CCK was not dose-dependent. DVZ suddenly suppressed amylase release 10 μM. This response was different from the chicken pancreatic acini stimulated with caerulein. In this case, DVZ suppressed amylase release in a dose response manner[17] and maximal inhibition was obtained at 10^{-6} M. It may be that the action of DVZ on inhibition of amylase secretion varies with the agonists, being better for caeruelin stimulation than CCK. Either way, a large dose of DVZ was required for inhibition of the action of CCK in amylase secretion in the present study.

It seems likely that the affinity of DVZ for CCK-A receptors is low in avian species compared with that in mammals. Most probably this is due to species differences in CCK-A receptor structure that influence antagonist binding, because a single amino acid change in the receptor structure can alter the effectiveness of non-peptide antagonists[19]. The cloning of the avian CCK receptor will help to clarify this possibility.

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