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Suppression of food intake induced by corticotropin-releasing factor family in neonatal chicks

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Abstract

Corticotropin-releasing factor (CRF), urocortin and urotensin I share amino acid sequences, and they inhibit food intake in mammals. CRF plays a potent role in decreasing food intake in avian species, but the effects of urocortin and urotensin I have not been investigated. Therefore, the effect of these three peptides on food intake in the neonatal chick was compared. In Experiment 1, birds were injected intracerebroventricularly (i.c.v.) with either 0, 0.01, 0.1 or 1 μg of urocortin following a 3-h fast, and food intake was measured for 2 h post-injection. Food intake was suppressed in a dose-dependent manner. Using a similar design in Experiment 2, the effect of urotensin I was investigated. Urotensin I appeared to suppress food intake in neonatal chicks more than urocortin did. In Experiment 3, the efficacy of CRF, urocortin and urotensin I was directly compared using one dose, 0.1 μg. The results indicated that the suppressive effect on food intake was strongest for CRF followed by urotensin I, then urocortin. These results suggest that the structure of receptors for the CRF family in chicks may be somewhat different than in mammals.

Keywords: Urocortin; Urotensin I; Corticotropin-releasing factor (CRF); Food intake; (Chick)

1. Introduction

Corticotropin-releasing factor (CRF), a peptide first isolated from mammalian brain, is a principal regulator of the hypothalamic–pituitary–adrenal axis and is suspected to play an important role in a variety of endocrine systems (Benoit et al., 2000; De Souza, 1987; Moreau et al., 1997). Another peptide, urotensin I, was subsequently isolated from the urophysis of teleost fish and shows close structural and biological homology with CRF and the frog skin peptide sauvagine (Britton et al., 1984; Lederis et al., 1982). Urocortin is a newly discovered 40-amino acid mammalian CRF-like peptide (Vaughan et al., 1995). It is structurally and pharmacologically similar to members of the CRF family of peptides. Urocortin was named for its sequence similarity to carp urotensin I and mammalian CRF (Koob, 1999; Vaughan et al., 1995).

Two receptors of the CRF family peptide have been identified, CRF_1 and CRF_2 receptor, with splice variants $CRF_{2\alpha}$ receptor, $CRF_{2\beta}$ receptor and $CRF_{2\gamma}$ receptor (Kostich et al., 1998; Lovenberg et al., 1995; Perrin et al., 1993). CRF and urocortin bind with high affinity to the CRF_1 receptor, but only urocortin binds with high affinity to the CRF_2 receptor, leading to a hypothesis that urocortin is the endogenous ligand for the CRF_2 receptor (Koob, 1999; Vaughan et al., 1995). In rats, urocortin binds 6 and 20 times more strongly than CRF to CRF_1 and CRF_2 receptors, respectively (Ohata et al., 2000).

CRF and urocortin have powerful behavioral and physiological effects when administered directly into the central nervous system. CRF injected intracerebroventricularly (i.c.v.) in doses of 0.01–0.10 μg produced electroencephalographic activation characteristic of arousal, and at higher doses produced seizure-like activity (Ehlers et al., 1983; Koob, 1999; Marrosu et al., 1987). Exogenously administered CRF produced behavioral activation, enhanced behavioral responses to stress, and a behavioral state that was aversive, resembling a state of stress (Aldenhoff et al., 1983; Dunn and Berridge, 1990).

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In addition to stress-related behaviors, CRF is thought to have an important role in the control of food intake and energy balance. Administration of exogenous CRF reduced food intake in rats (Benoit et al., 2000; Britton et al., 1984), mice (Contarino et al., 2000), chicks (Denbow et al., 1999; Furuse et al., 1997) and marsupials (Hope et al., 2000). It was reported that urocortin more potently reduced food intake than CRF under both fasted and ad libitum feeding conditions in mice (Contarino et al., 2000), rats (Ohata et al., 2000; Spina et al., 1996) and sheep (Parkes et al., 1997). This led to the hypothesis that the CRF_2 receptor is involved in the anorectic effects of CRF-related compounds. This hypothesis is supported by some preliminary antisense studies (Smagin et al., 1998). A comparison of the effects of these three different peptides on food intake in avian species has not been completed.

The aim of the present study was to identify the effects of urocortin and urotensin I on food intake. Furthermore, the order of potency of CRF, urocortin and urotensin I in inhibiting food intake in neonatal chicks was determined.

2. Materials and methods

Day-old male broiler chicks (Cobb; Mori Hatchery, Fukuoka, Japan) were housed in a windowless room at a constant temperature of 28 °C. Lighting was provided continuously. The birds were given free access to a commercial starter diet (Toyohashi Feed and Mills, Aichi, Japan) and water, and were maintained in accordance with recommendations of the National Research Council (1985). The birds were distributed into experimental groups based on their body weights so that the average body weight was as uniform as possible for each treatment. The brds were given the drugs (10 μl) i.c.v. using a microsyringe according to the method of Davis et al. (1979). In Experiment 1, after being deprived of food for 3 h, the birds (2-day-old, 10 birds/group) were injected with either 0, 0.01, 0.1 or 1 μg of urocortin, and cumulative food intake was measured for 0.5, 1 and 2 h. In Experiment 2, following 3 h of food deprivation, birds (3-day-old, 10 birds/group) were injected i.c.v. with either 0, 0.01, 0.1 or 1 μg of urotensin I.

Based on the results of Experiments 1 and 2, and previous results which showed that i.c.v. injections of CRF decreased food intake in neonatal chicks (Furuse et al., 1997), a third experiment was conducted to directly compare the efficacy of these three peptides. For this comparison, a dose of 0.1 μg of each peptide was chosen since this dose was shown to be effective in reducing food intake over a 2-h period in neonatal chicks. Therefore, in Experiment 3, the effect of i.c.v. injection of 0.1 μg of urocortin, urotensin I and CRF on food intake was compared in birds (2-day-old, 10 birds/group) deprived of food for 3 h before injection.

Rat urocortin and ovine CRF were purchased from Peptide Institute (Osaka, Japan) and urotensin I was purchased from Sigma (St. Louis, MO). The drugs were dissolved in a 0.1% Evans blue solution that was prepared

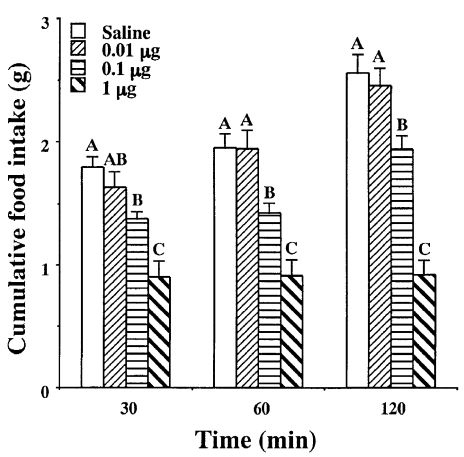

Fig. 1. Cumulative food intake of neonatal chicks injected i.c.v. with graded doses (0, 0.01, 0.1 and 1 μg) of urocortin after 3 h of fasting (Experiment 1). Means with a different letter are significantly different at $P < 0.05$. The following equations relating food intake to the urocortin dose (x, μg) were obtained. Food intake (g/30 min) = 1.725 (S.E. 0.087) − 3.880 (S.E. 1.541)x + 3.057 (S.E. 1.489)x^2 ($R^2 = 0.531$, $P <$ 0.0001), food intake (g/60 min) = 1.978 (S.E. 0.097) − 6.008 (S.E. 1.720)x + 4.941 (S.E. 1.661)x^2 ($R^2 = 0.605$, $P < 0.0001$) and food intake (g/120 min) = 2.538 (S.E. 0.106) − 6.534 (S.E. 1.874)x + 4.910 (S.E. 1.810)x^2 ($R^2 = 0.750$, $P < 0.0001$). The number of birds used was as follows: control, 7; 0.01 μg, 9; 0.1 μg, 10; and 1 μg, 10.

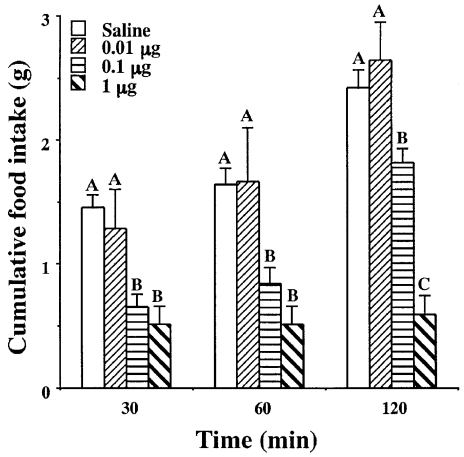

Fig. 2. Cumulative food intake of neonatal chicks injected i.c.v. with graded doses (0, 0.01, 0.1 and 1 μg) of urotensin I after 3 h of fasting (Experiment 2). Means with a different letter are significantly different at $P < 0.05$. The following equations relating food intake to the urotensin I dose (x, μg) were obtained. Food intake (g/30 min) = 1.420 (S.E. 0.121) − 8.486 (S.E. 2.116)x + 7.578 (S.E. 2.044)x^2 ($R^2 = 0.468$, $P <$ 0.0001), food intake (g/60 min) = 1.678 (S.E. 0.154) − 9.111 (S.E. 2.694)x + 7.946 (S.E. 2.601)x^2 ($R^2 = 0.446$, $P < 0.0001$) and food intake (g/120 min) = 2.536 (S.E. 0.133) − 7.676 (S.E. 2.317)x + 5.728 (S.E. 2.238)x^2 ($R^2 = 0.734$, $P < 0.0001$). The number of birds used was as follows: control, 9; 0.01 μg, 6; 0.1 μg, 10; and 1 μg, 9.

in 0.85% saline. The doses were prepared by repeated dilution with saline. Saline was used as a control in all experiments.

At the end of the experiment, the birds were anesthetized by injection of sodium pentobarbital and decapitated. The brains were removed and the location of the dye was confirmed. Data pertaining to individuals not found to have the dye present in the lateral ventricle were discarded. The data were analyzed by one-way analysis of variance by the general linear model procedure using a commercially available package (SAS Institute, 1985). Comparisons between means were made using Duncan's multiple range test. Regression equations with each time period were fitted to the data in Experiments 1 and 2. The results are presented as means ± S.E.M.

3. Results

The i.c.v. injection of urocortin suppressed food intake in a dose-dependent manner during the 2-h post-injection period (Fig. 1). Food intake was rapidly inhibited and injection of 1 μg of urocortin strongly inhibited food intake for 2 h post-injection. The inhibition of food intake by the injection of 0.01 and 0.1 μg of urocortin was attenuated over time. Similarly, central urotensin I strongly inhibited food intake in a dose-dependent fashion (Fig. 2). The effects of urotensin I were somewhat stronger than those of urocortin in Experiment 1. However, this effect could not be directly analyzed, therefore, the effects of CRF, urocortin and urotensin I on inhibition of food intake were compared directly in the final experiment. All three peptides significantly inhibited food intake compared with the saline control over 2 h (Fig. 3). Until 1 h, the biopotency to cause inhibition of food intake CRF > urotensin I > urocortin. Within the CRF family, the effect was significantly stronger for CRF than for urocortin. However, no significant differences within the CRF family were detected at 2 h.

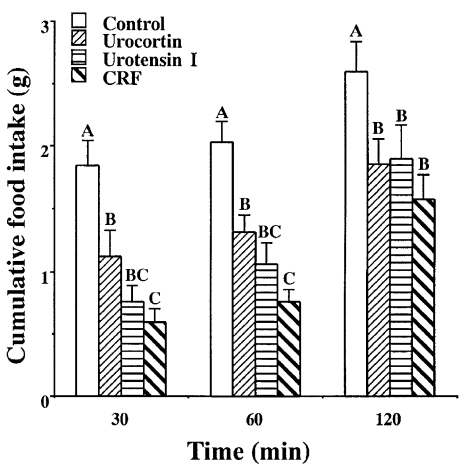

Fig. 3. Comparison of the effect (0.1 μg) on inhibition of food intake of corticotropin-releasing factor, urocortin and urotensin I in neonatal chicks. Means with a different letter are significantly different at $P < 0.05$. The number of birds used was as follows: control, 10; urocortin, 8; urotensin I, 9; and CRF, 10.

4. Discussion

It is generally accepted that peptides of the CRF family, including CRF, urocortin and urotensin I, are effective in decreasing food intake (Britton et al., 1984; Lederis et al., 1985; Spina et al., 1996). Although no comparisons of the behavioral effects of CRF, urocortin and urotensin I in chicks have been reported, Britton et al. (1984) showed that urocortin is more potent than CRF in inhibiting food intake in rats and CRF is more effective than urotensin I. CRF decreased food intake in both rats (Benoit et al., 2000; Britton et al., 1984) and chicks (Denbow et al., 1999; Furuse et al., 1997). However, there were some differences in CRF-induced behavior in the chick. For instance, the chicks in the CRF group displayed freezing behavior and moved less in a familiar environment during the early period of CRF treatment. Furthermore, CRF did not induce preening behavior in chicks (Ohgushi et al., 2001). These observations imply that the action of CRF itself or the contribution of CRF receptors may be different between mammal and avian species.

In rats, Koob and Heinrichs (1999) reported that urocortin has been identified in the brain and has a higher affinity for the CRF_2 receptor rather for the CRF_1 receptor. Urocortin has many of the effects of CRF but is also significantly more potent than CRF in decreasing feeding in both meal-deprived and free-feeding rats (Spina et al., 1996). Results to date have led to the hypothesis that the CRF_1 receptor may mediate CRF-like neuropeptide effects on behavioral responses to stressors, and that the CRF_2 receptor may mediate the suppression of feeding produced by CRF-like neuropeptides.

So far, no information about dose–response effects of urocortin and urotensin I on food intake is available for the chick. Thus, we confirmed the dose-related effects of urocortin and urotensin I in Experiments 1 and 2 by using three levels of both peptides. We previously obtained similar results with CRF (Furuse et al., 1997), in that the highest dose (1 μg) strongly inhibited food intake over 2 h. In addition, the effect of urocortin and urotensin I on food intake inhibition attenuated quickly with a dose of 0.01 μg and became extremely weak at 1 and 2 h. Therefore, to compare the food intake responses of all three peptides directly, the time course of changes in response to the intermediate dose (0.1 μg) of CRF, urocortin and urotensin I was compared in the same experiment. The results of Experiment 3 showed that in chicks CRF > urotensin I > urocortin in inhibiting food intake, which is different from results in mammals. The molecular weights of CRF, urotensin I and urocortin are 4670.4, 4869.5 and 4707.3, respectively. Thus, 0.1 μg of the three

peptides is almost equimolar (20.5–21.4 pmol). This suggests that the mechanism for the regulation of food intake in chicks is different from that in rats.

A possible explanation for this may be found in their different amino acid sequences. The greatest homology of amino acid sequences in the CRF family is found between urocortin (DDPPLSIDLTFHLLRTLLELARTQSQR ERAEQNRIIFDSV) and urotensin I (NDDPPISIDLTF HLLRNMIEMARIENEREQAGLNRKYLDEV) with 60% common amino acid residues. There is 42.5% (17 common amino acids) homology between CRF (SQEPP ISLDLTFHLLREVLEMTKADQLAQQAHSNRKLLDIA) and urocortin, and 52.5% (21 common amino acids) between CRF and urotensin I. The differences in four amino acid residues (21–17) between CRF and urocortin and urotensin I may influence the efficacy of the CRF family in the chick. In addition, the 10-amino acid residues (QAHSNRKLLD) starting from the third amino acid residue of the C-terminus of CRF is more similar to that of urotensin I (70%) than to that of urocortin (40%). It appears that in chicks the amino acid sequence of the C-terminus is possibly critical for binding with CRF receptors. This conclusion is consistent with that of Britton et al. (1984).

It has been proposed that the CRF_2 receptor plays a more important role than the CRF_1 receptor in decreasing food intake in rats, but this does not appear to be the case in chicks. Two possible hypotheses can be proposed to explain the difference in the effects on food intake between CRF and urocortin in chicks. One is that CRF may act through an unknown receptor that does not bind urocortin. The recently discovered $CRF_{2\alpha}$ receptor has no affinity for sauvagine and rat urocortin, but does bind rat and human CRF (Miyata et al., 1999). Chicks and rats possibly possess different CRF receptors, or at least the amino acid sequences of CRF receptors are not the same. Therefore, the potency order of these three peptides was not consistent with that in rats (Benoit et al., 2000). Another explanation is that inhibition of food consumption may occur by activation of neurons expressing the CRF_1 receptor. Furthermore, binding of urocortin to the CRF_2 receptor may block its affinity for other receptor site(s) of action, or these peptides may differ in their resistance to degradation. Our experiments lend some support to this later hypothesis since the action of urotensin I was slightly more rapidly attenuated than that of the other peptides 2 h post-injection (Fig. 3). It is suggested that the central action of the CRF family on food intake is different in chicks and mammals.

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