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The central corticotropin-releasing factor and glucagon-like peptide-1 in food intake of the neonatal chick

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Abstract

Recently, we have reported that central administration of glucagon-like peptide-1 (GLP-1) strongly decreases food intake of chicks. The aim of this study was to elucidate whether suppressed food intake induced by the central injection of GLP-1 is mediated by activation of the hypothalamic-pituitary-adrenal axis. First, the effects of central administration of corticotropin-releasing factor (CRF) were investigated. Birds (2-day-old) were food-deprived for 3 h and then CRF or saline was injected intracerebroventricular (i.c.v.). CRF strongly inhibited food intake. Thereafter, effects of central CRF or GLP-1 on plasma corticosterone concentration were examined. CRF significantly stimulated corticosterone release, but GLP-1 did not alter plasma corticosterone concentration. These results suggest that CRF is a potent inhibitor of food intake in the chick, but the suppression of food intake induced by central GLP-1 may not be involved in the activation of hypothalamic-pituitary-adrenal axis. © 1997 Elsevier Science B.V.

Keywords: GLP-1 (glucagon-like peptide-1); CRF (corticotropin-releasing factor); Hypothalamic-pituitary-adrenal axis; Feeding behavior; (Chicken)

1. Introduction

It is known that corticotropin-releasing factor (CRF), a 41-amino-acid peptide, has potent behavioral effects when administered intracerebroventricularly (i.c.v.) to rats. For instance, the rat grooms more (Britton et al., 1982; Morley and Levine, 1982), moves and rears less in the open field (Sutton et al., 1982), and moves more in a familiar environment (Britton et al., 1982; Morley and Levine, 1982; Sutton et al., 1982). Moreover, central CRF strongly inhibits food intake in the rat (Britton et al., 1982; Morley and Levine, 1982; Krahn et al., 1988). High concentrations of CRF immunoreactivities are found in cell bodies in the paraventricular nucleus of the hypothalamus and in the median eminence where the neurons originating in the paraventricular nucleus terminate (Olschowka et al., 1982; Cummings et al., 1983; Swanson et al., 1983). In the chicken, CRF immunoreactivity is also observed in the brain during incubation and after hatching (Józsa et al., 1986). To the authors' knowledge, no reports regarding the central effect of CRF on chicken feeding behavior have been published so far.

In mammalian species, proglucagon contains two glucagon-like sequences, glucagon-like peptide GLP-1 and GLP-2. In chickens, however, proglucagon does not contain GLP-2 (Hasegawa et al., 1990). Recently, GLP-1 has been shown to be related to feeding behavior; central administration of GLP-1 strongly inhibited food intake of rats (Tang-Christensen et al., 1996; Turton et al., 1996). We reported that central injection of mammalian and chicken GLP-1 similarly inhibited food intake in the chick (Furuse et al., 1997b). Moreover, effective level of GLP-1 was much lower in the chick than in the rat. However, the mechanism by which central GLP-1 suppresses food intake is not fully understood. According to Turton et al. (1996), c-*fos* appeared exclusively in the paraventricular nucleus

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of the hypothalamus and central nucleus of the amygdala following the i.c.v. GLP-1 injection. Neuropeptide Y is abundantly expressed throughout much of the central and peripheral nervous system. One of the most dramatic effect of neuropeptide Y is to induce food intake in satiated animals when administered i.c.v. (Clark et al., 1984; Stanley et al., 1986). In particular, injection of neuropeptide Y into the paraventricular nucleus produces a strong increase in food intake (Stanley and Leibowitz, 1985). The effect of neuropeptide Y on food intake is decreased by GLP-1 in the rat (Turton et al., 1996). Our data also confirmed that food intake enhanced by central neuropeptide Y was decreased, in a dose dependent fashion, by central GLP-1 in the chick (Furuse et al., 1997a). There is a stimulatory action of neuropeptide Y given intracisternally or by microinjection into the hypothalamus on the hypothalamic-pituitary-adrenal axis (Haas and George, 1987; Härfstrand et al., 1987; Wahlestedt et al., 1987). Wang et al. (1996) reported that i.c.v. injection of GLP-1 decreases food intake and increases the activity of the hypothalamic-pituitary-adrenal axis of rats. Thus, central GLP-1 may interact with CRF in the brain.

The purpose of the present study is to elucidate whether food intake of the chick is suppressed by the central CRF as observed in rats, and whether suppressed food intake by the central injection of GLP-1 is related with central CRF.

2. Materials and methods

Day-old broiler chicks of both sexes were purchased from a local hatchery (Fusoen, Aichi). The birds were maintained in a room with 24 h light and at a temperature of 28°C. They were given free access to a commercial starter diet (Nihon Nosan Kogyo Co., Tokyo) and were maintained in accordance with recommendation of the National Research Council (1985). The birds were distributed into experimental groups based on their body weight, so that an average body weight was as uniform as possible within the same experiment. After 3 h fasting, birds (2-day-old, 8–9 birds per group) were given the diet for 2 h immediately after administration of the peptides.

Immediately before receiving the diet, birds were fixed in a headholder (Davis et al., 1979) and injected with solutions (10 μl) using a microsyringe. Ovine CRF and chicken GLP-1-(7-36) were purchased from Peptide Institute, Inc. (Osaka, Japan). Peptides were dissolved in a 0.1% Evans Blue solution prepared in a 0.85% saline.

In experiment 1 birds were injected by the i.c.v. route with four levels (0, 1.25, 2.5 and 5 μg) of CRF and given the diet. Thereafter, one experiment was done by using lower levels of CRF; namely 0, 0.01, 0.1 and 1 μg in experiment 2. Food intake was determined at 30, 60 and 120 min. After confirming the strong effect of CRF on food intake, the effect of CRF on plasma corticosterone concentration was investigated in a further experiment. In the third experiment, CRF was applied at two levels (0 and 0.1 μg). After 30 and 60 min of administration, a blood sample was taken by heart puncture. The similar experiment was done by using chicken GLP-1 (0 and 0.03 μg) in experiment 4, because 0.03 μg of GLP-1 strongly inhibited food intake (Furuse et al., 1997a,b). Plasma corticosterone concentration was determined by the method described by Tanabe et al. (1986) using [1,2,6,7-^{3}H] corticosterone (DuPond, Wilmington, DE) and specific antibody for corticosterone (provided by Dr. R.J. Etches, University of Guelph, Canada). The radioimmunoassay of corticosterone was performed in a single assay. The intra-assay variation was 9.5% and the minimum standard concentration for corticosterone was 0.156 ng/ml.

At the end of the experiments, birds were killed by decapitation, followed by brain sectioning to identify location of drug injection. Data from the individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were deleted.

Data were subjected to one-way or two-way analysis of variance according the general linear model procedure using a commercially available package (SAS, 1985), and comparisons between means were made using Duncan's multiple range test or *t* test. The significance of difference between means for the various treatments in the cumulative food intake experiments was determined at each time at which food intake was measured. The results are shown as the means ± SEM.

3. Results

Fig. 1 shows cumulative food intake of birds injected i.c.v. with graded levels of CRF after 3 h fasting in

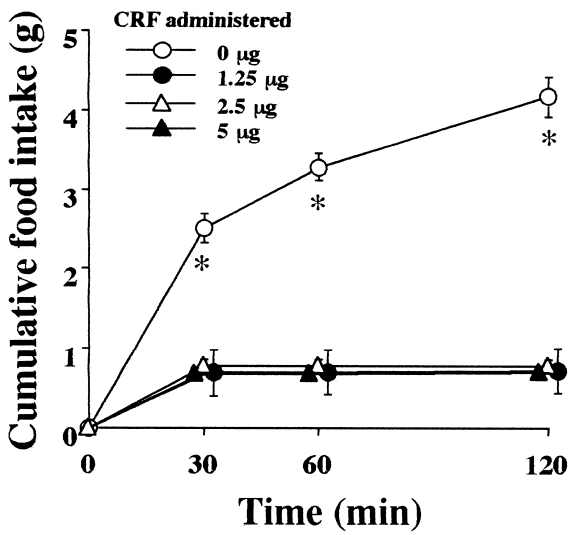

Fig. 1. Cumulative food intake of chicks injected i.c.v. with four levels of corticotropin-releasing factor (CRF; 0, 1.25, 2.5 or 5 μg per bird). * Significantly different at $P < 0.05$ compared with other groups at each time.

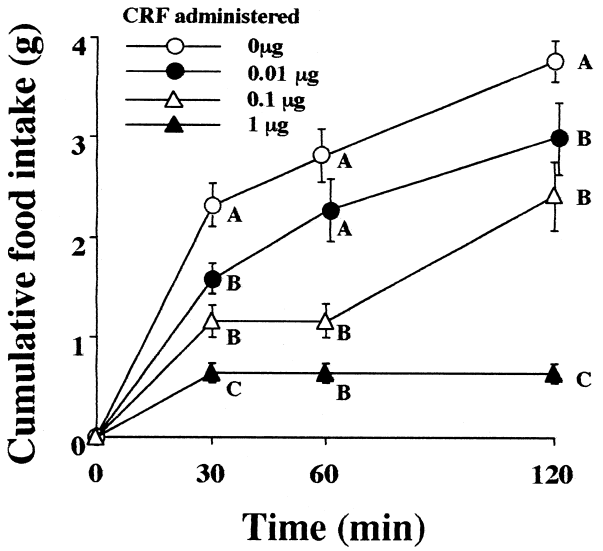

Fig. 2. Cumulative food intake of chicks injected i.c.v. with four levels of corticotropin-releasing factor (CRF; 0, 0.01, 0.1 or 1 μg per bird). Means with a different letter at each time are significantly different at $P < 0.05$.

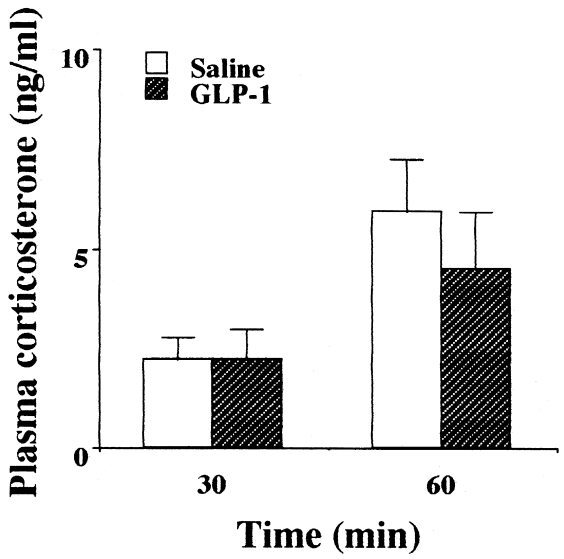

Fig. 4. Plasma corticosterone concentration after i.c.v. injection of saline or glucagon-like peptide-1 (GLP-1; 0.03 μg per bird) in the chick.

experiment 1. Food intake was rapidly inhibited by all doses of CRF. In this experiment, no dose-dependent relationship was observed. Thus, the lower doses (0.01, 0.1 and 1 μg) of CRF were applied in experiment 2. As shown in Fig. 2, CRF decreased food intake in a dose-dependent manner.

Fig. 3 shows plasma corticosterone concentration after i.c.v. administration of CRF. Central CRF significantly elevated plasma corticosterone concentration from the control level at both times determined. As demonstrated in Fig. 4, however, central GLP-1 did not alter plasma corticosterone concentration of chicks. Corticosterone concentrations at 60 min were significantly higher than those at 30 min.

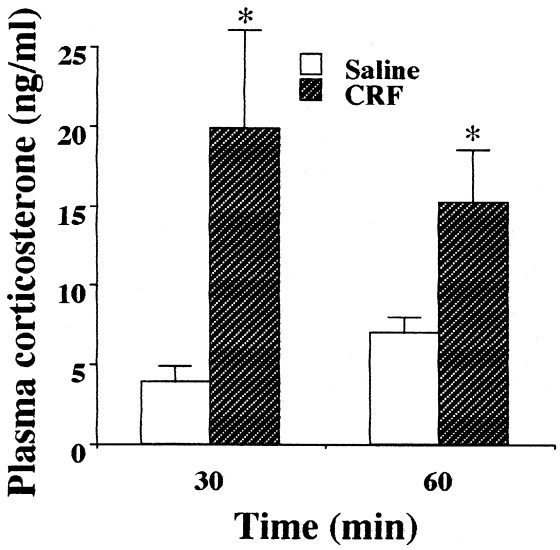

Fig. 3. Plasma corticosterone concentration after i.c.v. injection of saline or corticotropin-releasing factor (CRF; 0.1 μg per bird) in the chick. * Significantly different at $P < 0.05$ compared with corresponding controls.

4. Discussion

Birds treated with CRF ate some diet within the first 30 min, but thereafter would not eat any more (Fig. 1). This result implies that food intake is not completely inhibited by CRF immediately after its i.c.v. injection, but that the inhibition was detectable after 30 min and continued for at least 2 h after injection. The lower doses of CRF decreased food intake in a dose-dependent manner (Fig. 2), implying that central CRF may be the most potent inhibitor of food intake in the chicken.

That central CRF significantly elevated plasma corticosterone concentration implied that CRF activated the hypothalamic-pituitary-adrenal axis of chickens (Fig. 3). However, central GLP-1 did not alter plasma corticosterone concentration of chicks (Fig. 4). The reason for the increase in the plasma corticosterone concentration at 60 min (Fig. 4) was unclear, but the differences were very small. The values for the saline control groups in both third and fourth experiments were almost identical. Not only CRF (Fig. 2) but GLP-1 applied here (0.03 μg) markedly reduced the food intake of chicks over 2 h (Furuse et al., 1997a,b). Wang et al. (1996) reported that i.c.v. injection of GLP-1 increased plasma corticosterone and suggested that there was a relationship between suppressed food intake and the activities of the hypothalamic-pituitary-adrenal axis of rats. In contrast with findings for the rat, the present results suggest that central GLP-1 does not stimulate the hypothalamic-pituitary-adrenal axis in the chick. In rats, central CRF induces increased grooming

(Britton et al., 1982; Morley and Levine, 1982), less moving and rearing in the open field (Sutton et al., 1982), and move movement in a familiar environment (Britton et al., 1982; Morley and Levine, 1982; Sutton et al., 1982). In the present study, chicks also excited by central CRF moved more and vocalized loudly. However, the effect of central GLP-1 on behavior was completely different from the effect of central CRF, because the chicks were very calm and moved less after i.c.v. administration of GLP-1. Taken these results together, it is concluded that the mechanism by which central GLP-1 suppresses food intake may not involve the hypothalamic-pituitary-adrenal axis in relation to the activation of CRF release and may be different in rats and in chicks. For further clarification, however, studies using a CRF receptor antagonist remain to be done.

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