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# Interaction of corticotropin-releasing factor and glucagon-like peptide-1 on behaviors in chicks

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**Abstract**

Both corticortropin-releasing factor (CRF) and glucagon-like peptide-1 (GLP-1) inhibit food intake of chicks, but they also produce other behaviors. The present experiments were undertaken to clarify the interaction of CRF and GLP-1 regarding their anorectic actions as well as other behaviors. In Experiment 1, birds were injected intracerebroventricularly (i.c.v.), following a 3-h fast, with either saline, 0.1 μg of CRF, 0.1 μg of CRF + 0.1 μg of GLP-1 or 0.1 μg of CRF + 1 μg of GLP-1, and food intake was measured for 2 h. The injection of CRF decreased food intake, and CRF injected with GLP-1 suppressed food intake for up to 2 h. Birds were treated similarly in Experiment 2 in which the doses of CRF and GLP-1 were reversed. GLP-1 strongly suppressed food intake, and this effect was augmented by coadministration of CRF. In Experiment 3, the behaviors of chicks injected with saline, CRF (0.1 μg), GLP-1 (0.1 μg) or CRF (0.1 μg) + GLP-1 (0.1 μg) were monitored for the numbers of steps, vocalization and locomotion. Chicks were excited, moved more and vocalized loudly following injection of CRF, whereas an opposite response was seen with GLP-1. The behaviors were intermediate following the coinjection of the two peptides. In conclusion, CRF and GLP-1 interact in the chick brain, but the response depends on the behavior being measured. 

*Keywords:* CRF (Corticotropin-releasing factor); GLP-1 (Glucagon-like peptide-1); Food intake; Behavior; (Chick)

## 1. Introduction

Corticotropin-releasing factor (CRF) is a key regulator of brain excitability changes associated with stress (Ehlers et al., 1983). CRF has been shown to activate the sympathetic nervous system and also to produce behavioral activation such as increased locomotor activity, induction of aggression and enhancement of arousal (Shibasaki et al., 1991; Sutton et al., 1982). Centrally administered CRF is capable of producing significant elevations of plasma adrenocorticotropic hormone, epinephrine and norepinephrine concentrations (Brown et al., 1982). 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 CRF reduces food intake in rats (Benoit et al., 2000; Britton et al., 1982), mice (Contarino et al., 2000), chicks (Denbow et al., 1999; Furuse et al., 1997b) and marsupials (Hope et al., 2000).

Glucagon-like peptide-1 (GLP-1) is a potent glucose-dependent insulinotropic hormone which has important actions on gastric motility, suppression of plasma glucagon levels, and possibly on the promotion of satiety and stimulation of glucose disposal in peripheral tissues, independent of the actions of insulin (Kieffer and Habener, 1999). GLP-1-immunoreactive nerve fibers and terminals are widely distributed throughout the brain, with the highest density occurring in the hypothalamus, thalamus and septal regions (Drucker and Asa, 1988; Kreymann et al., 1989; Larsen et al., 1997a,b; Salazar and Vaillant, 1990; Shimizu et al., 1987). GLP-1 is considered a prospective drug for obesity because some investigators showed that GLP-1 effectively suppresses food intake in rats (Meeran et al., 1999; Tang-Christensen et al., 1996; Turton et al., 1996),

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chicks (Furuse et al., 1997a,b) and humans (Naslund et al., 1998).

When studying the interaction between CRF and GLP-1 in the rat brain, Larsen et al. (1997b) suggested that GLP-1 caused anorexia by activating the hypothalamic–pituitary–adrenocortical axis. This may occur through stimulation of CRF neurons, and this activation may be responsible for the inhibition of feeding behavior. Plasma corticosterone increased rapidly after central administration of GLP-1 in the rat. However, the mechanism by which central GLP-1 inhibits food intake of the chick is different from that in the rat, since central administration of GLP-1 did not alter plasma corticosterone concentration (Furuse et al., 1997b). Furthermore, chicks excited by central CRF had increased activity and vocalized loudly, whereas following central injection of GLP-1, the chicks were very calm and moved less (Furuse et al., 1997b). The interaction between CRF and GLP-1 in the chick brain is still obscure. The present study was done to clarify the interrelationships between CRF and GLP-1 regarding several behaviors in chicks.

## 2. Materials and methods

### 2.1. Experiment of food intake

A day-old male broiler chicks (Cobb; Mori Hatchery, Fukuoka, Japan) were individually housed in a cage (185 × 165 × 145 mm, every five cages connected as one unit) 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 weight so that the average body weight was as uniform as possible for each treatment. The birds were given the drugs intracerebroventricularly (i.c.v.) in a volume of 10 μl using a microsyringe according to the method of Davis et al. (1979). In Experiment 1, the birds (3-day-old, 10 birds/group) were injected with either saline, 0.1 μg of CRF, 0.1 μg of CRF + 0.1 μg of GLP-1 and 0.1 μg of CRF + 1 μg of GLP-1 after being deprived of food for 3 h. Cumulative food intake was then measured at 0.5, 1 and 2 h postinjection. In Experiment 2, a similar design was used, but the treatments were saline, 0.1 μg of GLP-1, 0.1 μg of GLP-1 + 0.1 μg of CRF and 0.1 μg of GLP-1 + 1 μg of CRF in neonatal chicks (2-day-old, 10 birds/group).

Ovine CRF and chicken GLP-1-(7–36) were purchased from Peptide Institute (Osaka, Japan). Drugs were dissolved in a 0.1% Evans Blue solution which was prepared 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 killed by an overdose of sodium pentobarbital. 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.

### 2.2. Behavioral experiments

A total of 40 male broiler chicks (a day-old Cobb; Mori Hatchery) were reared in this experiment. On the experimental day, chicks (2-day-old or 3-day-old) were injected i.c.v. with 0.1 μg of CRF, or 0.1 μg of GLP-1 or 0.1 μg of CRF + 0.1 μg of GLP-1. Then, to avoid disturbing the birds, individual chicks were placed into a 400 × 300 × 190 mm acrylic glass recording chamber in which the floor was divided into 12 identical squares. Video cameras and tape recorders were positioned to record in three different directions, and each animal was automatically monitored for 15 minutes. Following the completion of the experiment, the brain was removed and the location of the dye in the lateral ventricle was confirmed. Behaviors were classified into one of seven mutually exclusive categories: step, vocalization and locomotion (the number of entries into the squares was scored during the 15-min observation period), sitting/standing time, time eyes open/closed, stepping, jumping and preening.

### 2.3. Statistic analysis

Data were subjected to two-way analysis of variance with the General Linear Model Procedure, using a commercially available package (SAS, 1985). To compare the differences between means for behaviors, the chicks were considered as main plots and time after injection as subplots.

## 3. Results

Fig. 1 shows the cumulative food intake of chicks after i.c.v. injection with saline, CRF, or CRF with two doses of GLP-1 over 2 h. Significant effects of peptides ($F(3,28) = 24.39$, $P < 0.0001$) and time ($F(2,56) = 44.34$, $P < 0.0001$) were detected. A significant ($F(6,56) = 18.37$, $P < 0.0001$) interaction between peptides and time was also detected. Food intake was rapidly suppressed by peptide treatments compared with the control. The inhibition of food intake was stronger when CRF and GLP-1 were given together. The effect of GLP-1 was dose-dependent.

The effect of i.c.v. injection of saline, GLP-1 alone or GLP-1 with two doses of CRF on food intake is shown in Fig. 2. The effects of peptides ($F(3,31) = 48.50$, $P < 0.0001$) and time ($F(2,62) = 29.82$, $P < 0.0001$) were significant. A significant ($F(6,62) = 8.97$, $P < 0.0001$) interaction between peptides and time was detected. Food

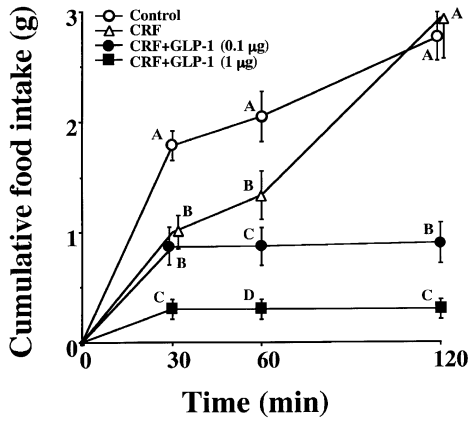


Fig. 1. Cumulative food intake of the neonatal chick injected i.c.v. with either saline, CRF (0.1 μg), CRF (0.1 μg)+GLP-1 (0.1 μg) or CRF (0.1 μg)+GLP-1 (1 μg) after 3-h fasting in Experiment 1. Values are means±S.E.M. Means with a different letter are significantly different with $P < 0.05$. The number of birds used was as follows: control, 8; CRF, 9; CRF+GLP-1 (0.1 μg), 7; and CRF+GLP-1 (1 μg), 8.

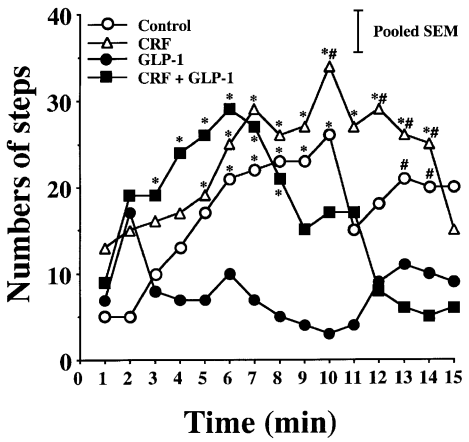


Fig. 3. Stepping of the neonatal chick injected i.c.v. with either saline, CRF (0.1 μg), GLP-1 (0.1 μg) or CRF (0.1 μg)+GLP-1 (0.1 μg) in Experiment 3. *, Significantly different from the GLP-1 group at $P < 0.05$. #, Significantly different from the CRF+GLP-1 group with $P < 0.05$. The number of birds used was as follows: control, 10; CRF, 10; GLP-1, 9; and CRF+GLP-1, 9.

intake was quickly decreased by peptide treatment and the effect of GLP-1 was reinforced by CRF in a dose-dependent fashion. No significant difference in food intake was detected between GLP-1 with 0.1 μg CRF and 1 μg CRF alone at 30 and 60 min, but the effect in the former group was somewhat attenuated at 120 min.

Fig. 3 shows the number of steps at each minute after i.c.v. injection with CRF, GLP-1, or both. The effects of the peptides were not significant ($F(3,34) = 1.21$, $P > 0.05$), but a significant ($F(14,476) = 1.84$, $P < 0.05$) time effect was detected. There was a significant ($F(42,476) = 1.45$, $P < 0.05$) interaction between peptide and time. Compared to the control, CRF-treated birds stepped more, whereas GLP-1-treated birds stepped less. The birds injected with both CRF and GLP-1 increased the number of steps during the first half of the observations, but the value gradually decreased to the value of the GLP-1 alone group.

The number of vocalizations at each minute is shown in Fig. 4. The effects of peptide ($F(3,34) = 17.06$, $P < 0.0001$) and time ($F(14,476) = 12.36$, $P < 0.0001$) were significant. A significant interaction ($F(42,476) = 2.05$, $P < 0.001$) was also detected. Birds injected with CRF vocalized with a high frequency during the entire experimental period. The number of vocalizations decreased rapidly in the GLP-1 group and was the lowest of all groups after 2 min. The birds receiving both peptides vocalized at an intermediate level, which was similar to that of the control group.

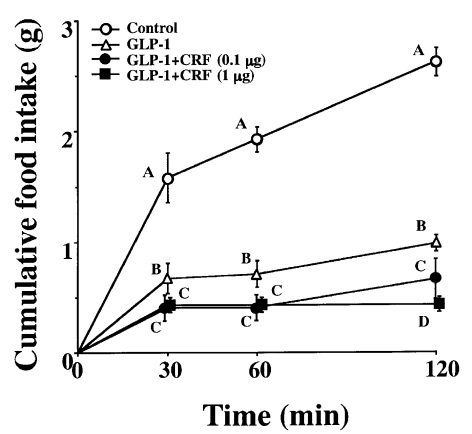


Fig. 2. Cumulative food intake of the neonatal chick injected i.c.v. with either saline, GLP-1 (0.1 μg), GLP-1 (0.1 μg)+CRF (0.1 μg) or GLP-1 (0.1 μg)+CRF (1 μg) after 3-h fasting in Experiment 2. Values are means±S.E.M. Means with a different letter are significantly different with $P < 0.05$. The number of birds used was as follows: control, 9; GLP-1, 9; GLP-1+CRF (0.1 μg), 8; and GLP-1+CRF (1 μg), 9.

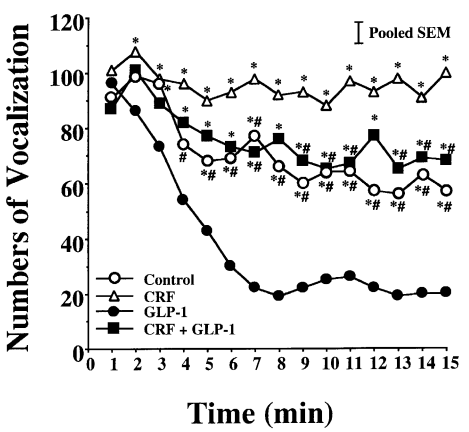


Fig. 4. Vocalization of the neonatal chick injected i.c.v. with either saline, CRF (0.1 μg), GLP-1 (0.1 μg) or CRF (0.1 μg)+GLP-1 (0.1 μg) in Experiment 3. *, Significantly different from the GLP-1 group with $P < 0.05$. #, Significantly different from the CRF group with $P < 0.05$. The number of birds used was as follows: control, 10; CRF, 10; GLP-1, 9; and CRF+GLP-1, 9.

Results for locomotion (not shown) correlated ($r = 0.954$) well with the results for stepping. No significant effects were detected on other behavioral parameters.

## 4. Discussion

The results for food intake are in good agreement with previous reports since CRF (Benoit et al., 2000; Britton et al., 1982; Contarino et al., 2000; Denbow et al., 1999; Furuse et al., 1997b; Hope et al., 2000) and GLP-1 (Donahey et al., 1998; Furuse et al., 1997a,b; Turton et al., 1996) both strongly inhibit food intake. However, the duration of inhibition of food intake was longer following GLP-1 than CRF. The effect of CRF alone disappeared at 2 h (Fig. 1), but continued after 2 h following GLP-1 alone (Fig. 2). The effect of each peptide was enhanced when it was injected in combination with the other peptide. This effect was shorter-lasting when CRF was added to GLP-1 (Fig. 2) since the response to GLP-1 plus 0.1 μg of CRF was somewhat attenuated at 2 h. On the other hand, GLP-1 strongly promoted the suppressive effect of CRF on food intake over 2 h when GLP-1 was added to CRF (Fig. 1).

There could be several reasons for the different response to each peptide. First, doses injected were on an absolute weight basis. The molecular weights of CRF and GLP-1 are 4670 and 3328, respectively. Therefore, a 0.1-μg dose of GLP-1 (30.0 pmol) was about 1.4 times higher than one of CRF (0.1 μg = 21.4 pmol) on a molar basis. However, the difference in responses between the two peptides could not be explained by this difference because the 2-h food intake did not return to its control value when a low dose (10 pmol) of GLP-1 was given (Furuse et al., 1997a). CRF-containing perikarya were found in the paraventricular nucleus of the hypothalamus of the brain of domestic fowl (Jozsa et al., 1984). A high concentration of both CRF (Dunn and Berridge, 1990) and GLP-1 (Larsen et al., 1997a) immunoreactive nerve fibers and terminals can be found in the paraventricular nucleus of the hypothalamus of rat. The paraventricular nucleus of the hypothalamus is also an important site for food intake control in chickens (Denbow and Sheppard, 1993) and rats (Tang-Christensen et al., 1996). GLP-1 may diffuse to the paraventricular nucleus of the hypothalamus faster than CRF due to the difference in molecular weight. This would, however, not explain the longer duration of response to GLP-1.

Second, the CRF used was not of chicken origin. Ovine CRF may bind loosely to the chicken CRF receptor. According to Yu et al. (1996), the chicken CRF receptor complementary DNA encodes a 420 amino acid protein that is 87–88% identical to those of human, rat and mouse. These authors also reported that urotensin I bound to the chicken CRF receptor with an apparent affinity 20 times greater than that of CRF and concluded that the chicken CRF receptor cloned by them was a type A CRF receptor. However, our previous data suggested that the inhibitory effect on food intake was stronger for ovine CRF than for urotensin I in neonatal chicks when administered i.c.v. (Zhang et al., 2001). Future studies using chicken CRF are necessary to clarify this issue, but to the authors' knowledge, chicken CRF has not yet been cloned.

Third, the half-lives of CRF and GLP-1 are different. The half-life of ovine CRF (Saphier et al., 1992; Schulte et al., 1982; Tsukada et al., 1984) was considerably longer than that of mammalian GLP-1 (Orskov et al., 1993; Schjoldager et al., 1989). Although the half-life of chicken GLP-1 has not been determined, Furuse et al. (1997a) reported that both mammalian and chicken GLP-1 inhibited food intake over 2 h in the neonatal chick, and no significant differences were observed between GLP-1 from two different species. Based on these facts, the effect of CRF on food intake should last longer than that of GLP-1 in neonatal chicks, but the present data do not support this idea.

CRF acted synergistically with GLP-1 to inhibit food intake. However, such an effect was not evident for other behaviors. For example, the combination of CRF and GLP-1 caused an intermediate effect between that of CRF alone and of GLP-1 alone on both stepping (Fig. 3) and vocalization (Fig. 4). In rats, central CRF induces increased grooming (Britton et al., 1982; Morley and Levine, 1982). CRF decreases moving and rearing in the open field (Sutton et al., 1982), but increases moving in a familiar environment (Britton et al., 1982; Morley and Levine, 1982; Sutton et al., 1982).

Furuse et al. (1997b) reported that chicks were excited by central CRF, as evidenced by increased movement and loud vocalization. In contrast, 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. Chicks differ from rats in some of the behavioral responses to neuropeptides. For instance, Furuse et al. (2001) reported that i.c.v. injection of ghrelin and growth hormone-releasing factor inhibits food intake in neonatal chicks. These findings are completely different from those for mammals. The action of GLP-1 may have a different action on behavior in rats and in chicks. According to Larsen et al. (1997b), central administration of either CRF or GLP-1 significantly stimulates corticosterone release in rats. In chicks, however, GLP-1 fails to stimulate corticosterone release (Furuse et al., 1997b). It was shown that the effect of CRF on food intake inhibition did not result from general malaise since water intake was not inhibited following similar injections in chicks (Denbow et al., 1999). Recently, Contarino et al. (2000) showed that motor activation elicited by CRF did not seem to account for the feeding results because no locomotor response to CRF was detected in CRFR1$^{-/-}$ mice, although the mice displayed prominent food intake suppression. GLP-1 was considered a potent physiological regulator of satiety based on its

effect to reduce food intake (Campos et al., 1994; Hoosein and Gurd, 1984; Turton et al., 1996; Van Dijk et al., 1997). Furthermore, Tang-Christensen et al. (1996) showed that GLP-1-induced anorexia cannot be attributed to inhibition of motor activity in rats. GLP-1 has been reported to reduce distress vocalization, whereas CRF has an opposite effect (Panksepp et al., 1997). CRF increases stepping of neonatal chicks (Ohgushi et al., 2001), but GLP-1 induces sleep-like behavior and reduces locomotion (Bungo et al., 1999a). Thus, in the chicken brain, CRF and GLP-1 have different effects on behaviors such as vocalization and locomotion.

The finding of different actions of CRF and GLP-1 on behaviors may be explained by the following facts. According to Bungo et al. (1999b), although a high dose of clonidine, an $\alpha_2$-adrenoreceptor agonist, induces a narcoleptic response and reduces food intake, food intake is enhanced by lower doses of clonidine. Rossi et al. (1983) reported that clonidine reduces distress vocalization of the chick. We have found that although central administration of GLP-1 significantly decreased food intake, the anorexigenic effect of GLP-1 was attenuated by fusaric acid, an inhibitor of dopamine-β-hydroxylase which is a rate-limiting enzyme of norepinephrine synthesis (Bungo and Furuse, in press). If GLP-1 stimulates the production and/or release of norepinephrine, the data obtained for both feeding behavior and other behaviors are not contradictory. On the other hand, the possibility that the discrepant actions of CRF and GLP-1 may be due to the different ability of the peptides to activate different brain neurotransmitter functions. For instance, there could be an action of CRF mediated by the catecholamines (Smagin et al., 1995) and one of GLP-1 mediated by activation of serotoninergic pathways since GLP-1 induced a satiety and sleep-like behavior (Bungo and Furuse, 2001; Bungo et al., 1999a). Further studies will be needed to clarify the central action of CRF and the adrenergic and serotoninergic systems in the chick. In conclusion, CRF and GLP-1 act synergistically within the central nervous system of neonatal chicks to reduce food intake, but appear to act antagonistically for other behaviors induced by stress.

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