Central administration of glucagon suppresses food intake in chicks

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Keywords:
Food intake, Chicken, Glucagon, Hyperglycemia

Abstract

Food intake in chickens is regulated in a manner similar to that in mammals. Corticotropin-releasing factor (CRF), which increases the plasma corticosterone concentration, plays an important role as a mediator of many appetite-suppressive peptides in the central nervous system in both species. Central administration of glucagon suppresses food intake in rats. However, the anorexigenic action of glucagon in chicks has not yet been identified. In the present study, we investigated the effects of central administration of glucagon on food intake in chicks. Intracerebroventricular administration of glucagon in chicks significantly suppressed food intake and significantly induced hyperglycemia. In contrast, peripheral administration of the same dose of glucagon did not influence food intake and plasma glucose concentration. These results suggest that glucagon functions in chicks as an appetite-suppressive peptide in the central nervous system. Intracerebroventricular administration of glucagon in chicks also significantly increased CRF mRNA expression and plasma corticosterone concentration, suggesting that CRF acts as a downstream molecule for a glucagon-induced appetite-suppressive pathway in chicks. It is likely that the induction of hyperglycemia by central administration of glucagon is involved in its anorexigenic action, because peripheral administration of glucose in chicks suppressed food intake. These results suggest that CRF- and/or hyperglycemia-mediated pathways are involved in the anorexigenic action of glucagon in chicks.

The regulation of food intake in poultry has been a focus of research interest in recent decades [8, 18], because growth rate and meat production can be improved by increased food intake. Food intake in chickens is regulated in a manner similar to that in mammals in many respects. For example, mammals and chickens have a similar regulatory system of food intake in the central nervous system [8], and corticotropin-releasing factor (CRF) acts as a mediator of a number of anorexigenic peptides in both chickens [14, 26, 27] and mammals [5, 7, 20, 28, 30]. In mammals, the central regulatory system of food intake is closely related to energy stores and fuel metabolism. Peripheral peptide hormones such as leptin and insulin are sensed by the hypothalamus, which, in turn, modulates food intake and energy availability [21]. Glucagon, one of the pepripheral peptide hormones, plays important roles in energy homeostasis [29]. Central administration of glucagon in rats suppresses food intake [6]. In chickens, glucagon-family peptides such as glucagon-like peptide-1 [27], pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide [26] can suppress food intake. Glucagon receptors exist in avian brains [15]. It is therefore likely that glucagon functions as an anorexigenic peptide in chickens. However, the effect of central administration of glucagon on food intake in chickens has not yet been identified.

In this study, we focused on the functional roles of glucagon in the central regulation of food intake in chicks. The results show direct evidence that glucagon suppresses food intake in the central nervous system in chicks.

Day-old male chicks (White Leghorn) were purchased from a local hatchery (Ghen Corporation, Gifu, Japan). They were given free access to water and a commercial chick starter diet (Nippon Formula Feed Mfg. Co., Ltd., Kanagawa, Japan). Room temperature was maintained at 32°C ± 2°C. All experimental procedures followed the guidelines for the care and use of experimental animals at the Rokkodai Campus of Kobe University in Japan. Chicken glucagon was purchased from Peptide Institute, Inc. (Osaka, Japan). All primers were purchased from Hokkaido System Science Co., Ltd. (Hokkaido, Japan).

Initially, we carried out a screening experiment to examine the effect of central administration of glucagon (dose: 0.3-3 μg) on food intake in chicks. We found that the central administration of glucagon suppressed food intake, and higher doses (1 and 3 μg) significantly suppressed food intake at similar levels (47.3% and 40.5%, respectively). Based on these results, doses from 0.1 to 1 μg were used in Experiment 1. In addition, the dose 1 μg, which significantly suppressed food intake throughout the experimental periods in Experiment 1, was used in Experiments 2-4.

In Experiment 1, 8-day-old chicks were divided into four groups. Glucagon was dissolved in a 0.85% (w/v) saline solution containing 0.1% (w/v) Evans Blue. Either glucagon (0.1, 0.3, or 1μg) or saline (as a control) was intracerebroventricularly administered according to the method of Davis *et al.* [3] at a volume of 10 μl after 3 h of fasting. Food intake was measured at 30, 60 and 120 min after administration. At the end of the experiment, the chicks were decapitated and their blood was collected. Plasma glucose

concentration was determined by the Somogyi-Nelson method [24]. Verification of injection was made by observation of the presence of Evans Blue dye in the lateral ventricle.

In Experiment 2, 8-day-old chicks were divided into two groups, and either 1 μg glucagon or saline (as a control) was administered via a wing vein at a volume of 200 μl after 3 h of fasting. Food intake was measured at 30, 60 and 120 min after administration. At the end of the experiment, the chicks were decapitated and their blood was collected. Plasma glucose was determined as described above.

In Experiment 3, 8-day-old chicks were divided into two groups, and either 1 μg glucagon or saline (as a control) was intracerebroventricularly administered as described in Experiment 1. Suda *et al.* reported that insulin-induced increase of hypothalamic CRF mRNA reached to the highest level at 120 min after administration in rats [25]. We followed this study and measured CRF mRNA level at 120 min after central administration of glucagon. At 120 min after administration, the chicks' brains were removed within 1 min of decapitation, frozen on powdered dry ice, weighed and stored at -80°C for further analysis. The hypothalamus was dissected from the frozen brain referring to a stereotaxic atlas drawn by Kuenzel and Masson [9] and weighed. Total RNA was extracted from the hypothalamus using the Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from 5 μg of total RNA treated with DNase I (Invitrogen, Carlsbad, California, USA) using the SuperScript III First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, California, USA) with random primers. PCR was performed using TaKaRa Ex Taq (Takara Bio, Inc., Siga, Japan). Chicken CRF cDNA (GenBank accession no. AJ621492) was amplified with the following primers: sense, 5'-CTC CCT GGA CCT GAC TTT-3'; antisense, 5'-CCT CAC TTC CCG ATG ATT-3'. As an internal standard, chicken 18S ribosomal RNA (GenBank accession no. AF173612) was also amplified, using the following primers: sense, 5'-CGC GTG CAT TTT ACA GAC CA-3'; antisense, 5'-ACC CGT GGT CAC CAT GGT A-3'. PCR reactions were performed as follows: 94°C for 5 min; 33 cycles (CRF) or 15 cycles (18S ribosomal RNA) of 94°C for 30 sec, 55°C for 1min and 72°C for 1 min; and final extension at 72°C for 4 min. The PCR products were analyzed by ethidium bromide (Nacalai Tesque, Inc., Kyoto, Japan) staining on 1% agarose gel. The fluorescence from PCR products was detected and quantified with Typhoon 9400 (Amersham Biosciences Corp., Piscataway, New Jersey, USA).

In Experiment 4, 8-day-old chicks were divided into two groups, and either 1 μg glucagon or saline (as a control) was intracerebroventricularly administered as described in Experiment 1. Culbert and Wells reported that peripheral administration of adrenocorticotropin (ACTH), which is a downstream mediator of CRF, increased plasma corticosterone concentration, and its highest level was observed at 120 min after ACTH administration in laying hens [1]. We followed this study and measured plasma corticosterone at 120 min after central administration of glucagon. At 120 min after administration, the chicks were decapitated and their blood was collected. Plasma corticosterone was determined by enzyme immunoassay using a commercial kit (Correlate-EIA, Assay Designs, Inc., USA).

In Experiment 5, 8-day-old chicks were divided into two groups, and either glucose (900 mg/kg body weight) or saline (as a control) was administrated via a wing vein at a volume of 3 ml/kg body weight after 3 h of fasting. Food intake was measured at 30, 60 and 120 min after administration.

Data from Experiment 1 were analyzed by the Tukey-Kramer test. Data from other experiments were analyzed by Student's *t* test. All statistics was performed using the commercial package (StatView version 5, SAS Institute, Cary, North Carolina, USA, 1998).

Central administration of glucagon in chicks significantly suppressed food intake ($p < 0.05$) and significantly increased plasma glucose concentration ($p < 0.05$) in a dose-dependent manner (Experiment 1, Figs. 1 and 2). In contrast, peripheral administration of the same dose of glucagon (1 μg) had no effect on food intake and plasma glucose concentration (Experiment 2, Table 1). These results suggest that glucagon functions in chicks as an appetite-suppressive peptide in the central nervous system. Smith and Bright-Taylor reported that peripheral administration of higher dose (200 μg/kg body weight) of glucagon suppresses food intake and increases plasma glucose concentration in chickens [23]. This dose is 16-fold higher than the dose we used in Experiment 2 (1 μg glucagon/birds is equal to 12.5 μg/kg body weight). However, such a high dose of glucagon may suppress food intake and increase plasma glucose concentration also in chicks.

CRF is a crucial mediator in the anorexgenic pathway of the central nervous system in mammals [5, 7, 20, 28, 30] and chickens [14, 26, 27]. Therefore, we next examined whether hypothalamic CRF acts as a downstream mediator of the glucagon-induced anorexigenic pathway in chicks. Glucagon administration significantly upregulated CRF mRNA expression ($p < 0.05$) (Experiment 3, Fig. 3). Since central administration of CRF increases plasma corticosterone concentration in chicks [19], we examined the effect of glucagon on plasma corticosterone concentration. Glucagon administration significantly increased plasma corticosterone concentration ($p < 0.05$) (Experiment 4, Fig.4). These data clearly demonstrated the involvement of CRF in the inhibitory mechanisms of glucagon for feeding in chicks.

It is noteworthy that central administration of glucagon significantly increased plasma glucose concentration ($p < 0.05$) (Experiment 1, Fig. 2). Intrahepatic administration of glucose has been shown to decrease food intake in cockerels [11, 22]. Therefore, we examined the effects of peripheral administration of glucose on food intake to evaluate whether the glucagon-induced hyperglycemia is involved in its anorexigenic action. We found that peripheral administration of glucose (dose: 150-1200 mg/kg body weight) increased plasma glucose concentration in chicks (data not shown). In particular, the increase of plasma glucose concentration was significant (350.2 ± 32.0 mg/dl) at 30 min when 900 mg/kg body weight glucose was administered, and this increase was similar to that of the glucagon-induced hyperglycemia (380.3 ± 31.8 mg/dl) observed in Experiment 1. Therefore, we used 900 mg/kg body weight glucose for peripheral administration and found that it significantly decreased food intake ($p < 0.05$) in chicks (Experiment 5, Fig. 5). Thus, our findings suggest that glucagon-induced hyperglycemia is associated with its anorexigenic action in chicks. Margolis *et al.* [12] showed that peripheral administration of the synthetic glucocorticoid dexamethasone in rats increases plasma glucose concentration. One report has shown that central administration of glucagon in rats induces hyperglycemia via autonomic nervous system [13]. It is

therefore likely that the hyperglycemia induced by central administration of glucagon in chicks occurs via the influence of corticosterone and/or via the autonomic nervous system.

In summary, we studied the functional roles of glucagon in the central regulation of food intake in chicks. We clearly demonstrated the direct evidence that central administration of glucagon significantly suppressed food intake in chicks. We suggest that CRF acts as a mediator in the downstream pathway of glucagon and that hyperglycemia is associated with the appetite-suppressive action of glucagon. These results suggest that glucagon functions in chicks as an appetite-suppressive peptide in the central nervous system.

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Figure legends

Fig. 1. Effect of central administration of glucagon on cumulative food intake in chicks. Data are means ± S.E.M. The number of chicks used is shown in parentheses. Groups with different letters are significantly different ($p < 0.05$) at each time point.

Fig. 2. Effect of central administration of glucagon on plasma glucose concentration in chicks. Data are means ± S.E.M. The number of chicks used is shown in parentheses. Groups with different letters are significantly different ($p < 0.05$).

Fig. 3. Effect of central administration of glucagon on hypothalamic CRF mRNA expression in chicks. Data are means ± S.E.M. of six chicks. *, significant with respect to saline ($p < 0.05$).

Fig. 4. Effect of central administration of glucagon on plasma corticosterone concentration in chicks. Data are means ± S.E.M. of five chicks. *, significant with respect to saline ($p < 0.01$).

Fig. 5. Effect of peripheral administration of glucose (900 mg/kg) on cumulative food intake in chicks. Data are means ± S.E.M. The number of chicks used is shown in parentheses. *, significant with respect to saline ($p < 0.05$) at each time point.

Table 1

Effects of peripheral administration of 1 μg glucagon on cumulative food intake and plasma glucose concentration in chicks

	Cumulative food intake (g)			Plasma glucose (mg/dl)
	30 min	60 min	120 min	
Saline	1.59 ± 0.26	2.48 ± 0.24	3.46 ± 0.32	291.6 ± 7.7
Glucagon	1.45 ± 0.40	2.25 ± 0.45	3.05 ± 0.56	284.8 ± 10.9

Values are means ± S.E.M. of eight chicks in each group.

Figure1
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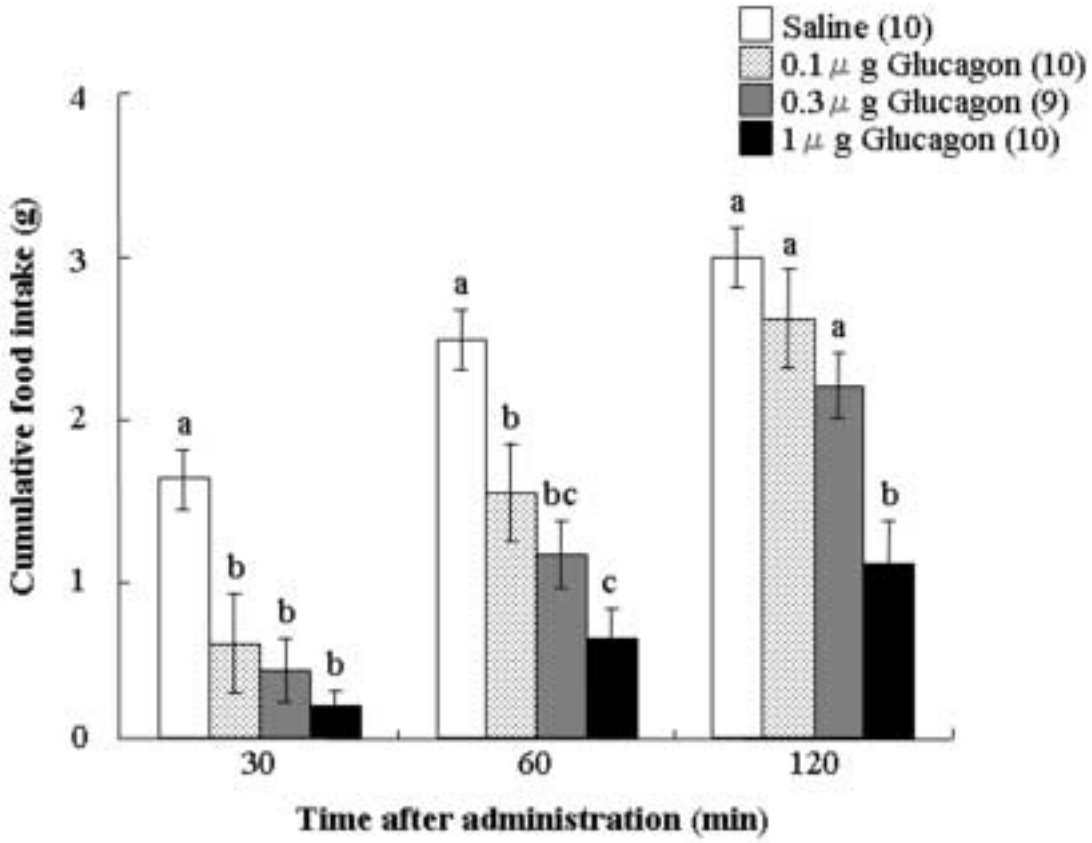

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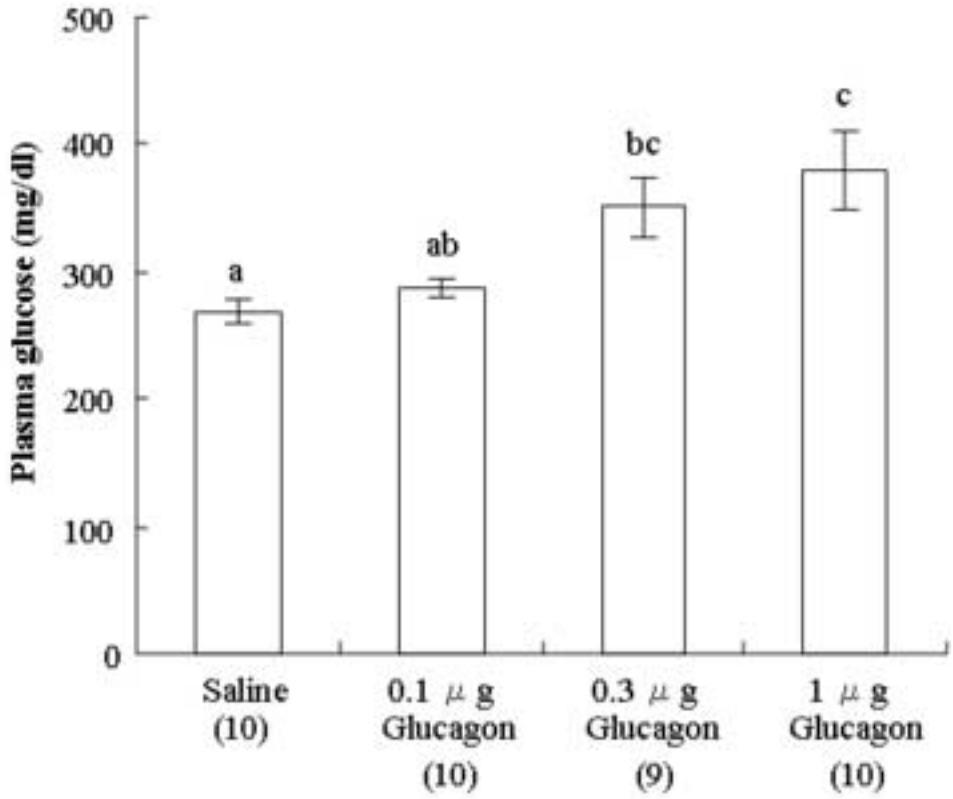

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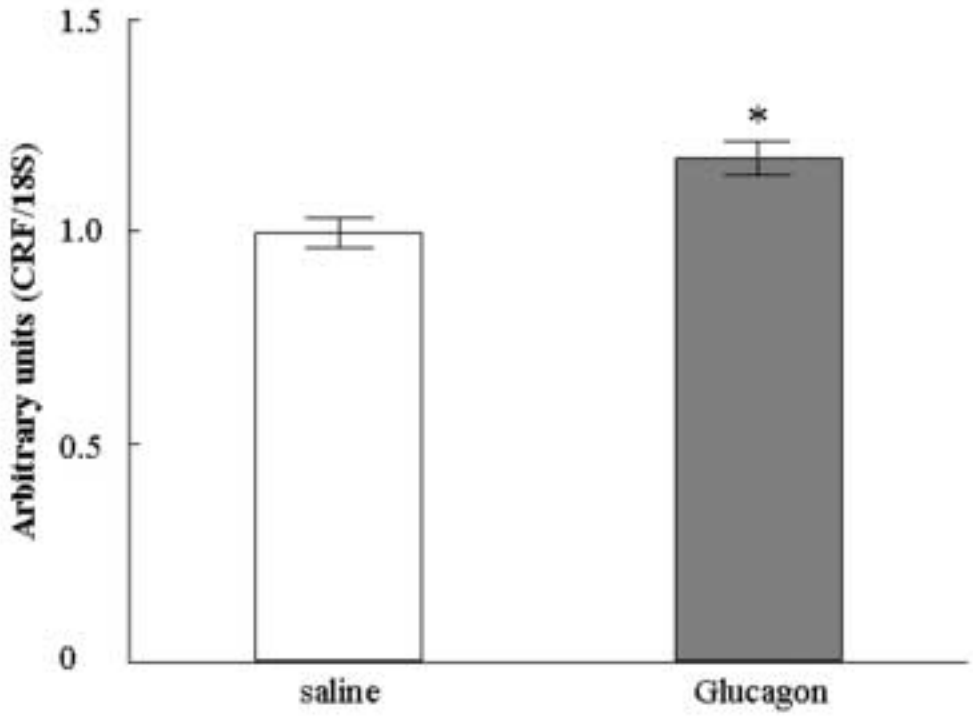

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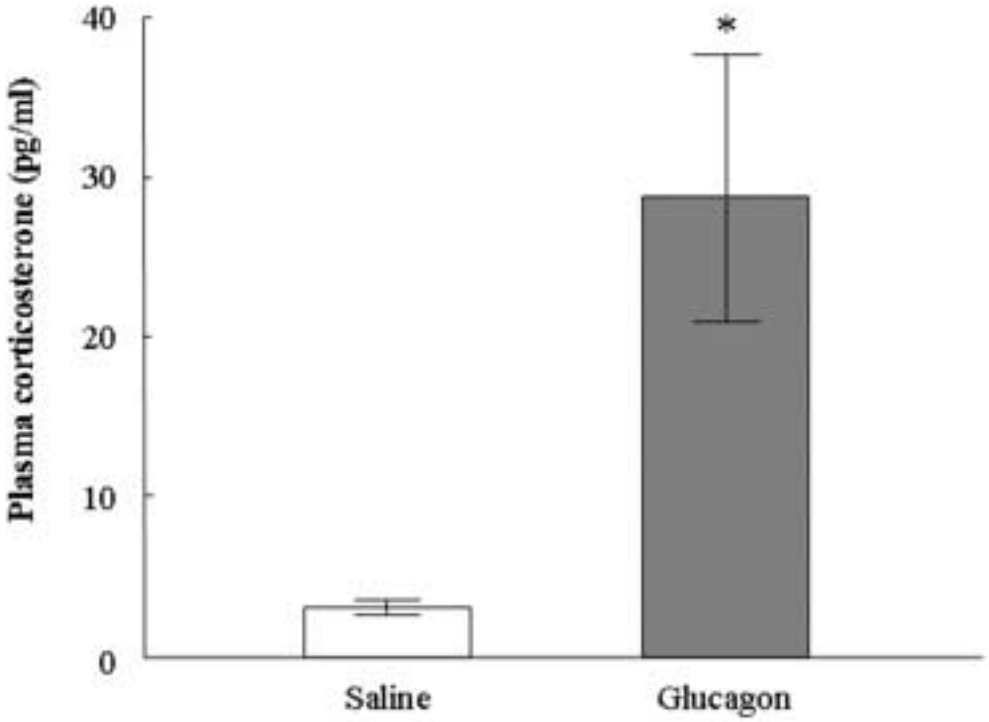

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