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Intracerebroventricular administration of novel glucagon-like peptide suppresses food intake in chicks

Kazuhisa Honda[a,*], Takaoki Saneyasu[a], Takuya Yamaguchi[a], Tomohiko Shimatani[b], Koji Aoki[b], Kiwako Nakanishi[a], Hiroshi Kamisoyama[a]

[a] Department of Bioresource Science, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan
[b] Faculty of Agriculture, Kobe University, Kobe 657-8501, Japan

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ABSTRACT

Glucagon-related peptides such as glucagon, glucagon-like peptide-1, and oxyntomodulin suppress food intake in mammals and birds. Recently, novel glucagon-like peptide (GCGL) was identified from chicken brain, and a comparatively high mRNA expression level of GCGL was detected in the hypothalamus. A number of studies suggest that the hypothalamus plays a critical role in the regulation of food intake in mammals and birds. In the present study, we investigated whether GCGL is involved in the central regulation of food intake in chicks. Male 8-day-old chicks (*Gallus gallus*) were used in all experiments. Intracerebroventricular administration of GCGL in chicks significantly suppressed food intake. Plasma glucose level was significantly decreased by GCGL, whereas plasma corticosterone level was not affected. Central administration of a corticotrophin-releasing factor (CRF) receptor antagonist, α-helical CRF, attenuated GCGL-suppressed food intake. It seems likely that CRF receptor is involved in the GCGL-induced anorexigenic pathway. All our findings suggest that GCGL functions as an anorexigenic peptide in the central nervous system of chicks.

1. Introduction

The regulatory mechanisms of food intake by neuropeptides and hormones in the central nervous system have been demonstrated in several species, including mammals [28], birds [2], amphibians [5], and fishes [47]. Glucagon-related peptides such as glucagon, glucagon-like peptide-1 (GLP-1), and oxyntomodulin (OXM) suppress appetite in mammals [9,18,23,41] and birds [4,14,15,27,34,40]. In chickens, central administration of glucagon, GLP-1, and OXM suppresses food intake in chicks [4,14,15,27,40]. There is evidence that central administration of glucagon and GLP-1 significantly increased plasma corticosterone levels, suggesting that both of them stimulate hypothalamic-pituitary-adrenal axis [15,40]. However, possible mechanisms underlying the anorexigenic actions of glucagon and GLP-1 were different. For example, the anorexigenic effect of GLP-1 could be attenuated by the co-administration of a CRF receptor antagonist astressin [40], whereas the effect of glucagon could not be attenuated by the co-administration of a CRF receptor antagonist α-helical CRF [15]. Central administration of glucagon significantly increased plasma glucose level, suggesting that the hyperglycemia is related to glucagon-induced anorexia [14]. In contrast, central administration of GLP-1 decreased plasma glucose level in chicks [37]. It is therefore likely that glucagon-related peptides suppress food intake in chicks by different mechanisms.

Recently, novel glucagon-like peptide (GCGL) and its receptor (GCGLR) were identified in chicken brain [49]. GCGL shares high amino acid sequence identity with chicken glucagon-related peptides (Fig. 1) [49]. Genomic analysis revealed that the GCGL gene is located in a synteny conserved in tilapia, coelacanth, *Xenopus*, and chicken, but not in human [49]. GCGL and GCGLR were detected in the brain of chicken and *Xenopus tropicalis* by RT-PCR [19,49], implying that GCGL and its receptor may play important roles in the central nervous system of chicken and other nonmammalian vertebrates. Furthermore, a comparatively high mRNA expression level of GCGL was detected in the hypothalamus of chickens [49]. A number of studies suggest that the hypothalamus plays a critical role in the regulation of food intake in vertebrates [2,5,28,47]. It is therefore possible that GCGL functions as an anorexigenic peptide in chicken hypothalamus. However, the physiological function of GCGL has not yet been identified.

In the present study, to investigate whether GCGL is involved in the central regulation of food intake in chicks, we examined (1) the

* Corresponding author at: Department of Bioresource Science, Graduate School of Agricultural Science, Kobe University, 1-1, Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan. Tel.: +81 78 803 5809; fax: +81 78 803 5809.
E-mail addresses: honda@tiger.kobe-u.ac.jp, kazuhisahonda2000@yahoo.co.jp (K. Honda).

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GCGL     : HSEGTFTSDFTRYLDKMKAKDFVHWLINT
Glucagon: --Q------YSK---SRR-Q---Q--MS-
OXM      : --Q------YSK---SRR-Q---Q--MS-KRNGQQGQEDKENDKFPDQLSSNAIS
GLP-1    : -A---Y---I-S--ESRR--E-IA--V-G
GLP-2    : -AD------INKI--D-A--E-LK-----KVTQ
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Fig. 1. Alignment of glucagon-related peptides in chickens. Dashes indicate amino acids identical to glucagon. Abbreviations are as follows: GCGL, novel glucagon-like peptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; OXM, oxyntomodulin.

effects of central administration of GCGL on food intake and plasma components in chicks; (2) the effects of the CRF receptor antagonist α-helical CRF on the GCGL-suppressed food intake in chicks; and (3) the effects of fasting on the mRNA levels of GCGL and GCGLR in the hypothalamus of chicks.

2. Materials and methods

2.1. Animals and peptides

Day-old male layer chicks (White Leghorn) and broiler chicks (Ross strain) were purchased from a local hatchery (Ghen Corporation, Gifu, Japan and Ishii Co. Ltd., Tokushima, Japan). They were given free access to water and a commercial chick starter diet (Nippon Formula Feed Mfg. Co., Ltd., Kanagawa, Japan) under 24-h lighting conditions. Room temperature was maintained at 32 ± 2 °C. This study was approved by the Institutional Animal Care and Use Committee (Permission number: 24-03-06) and carried out according to the Kobe University Animal Experimentation Regulation. Chicken GCGL was purchased from Funakoshi Co. Ltd. (Tokyo, Japan). α-helical CRF was purchased from Peptide Institute, Inc. (Osaka, Japan). All primers were purchased from Life Technologies Corporation (Carlsbad, CA, USA).

2.2. Experiment 1: effects of central administration of GCGL on food intake in chicks

Fifty six 8-day-old layer or broiler chicks were weighed and allocated to four groups based on body weight (14 birds in each group). GCGL was dissolved in a 0.85% (w/v) saline solution containing 0.1% (w/v) Evans Blue. The peptides were intracerebroventricularly administered according to the method of Davis et al. [10] at a volume of 10 μl after 3 h of fasting. Chicks were administered GCGL (0, 0.1, 0.3 or 1 nmol). Food intake was measured at 30, 60 and 120 min after administration. At the end of the experiment, the chicks were sacrificed by decapitation. Verification of injection was made by observation of the presence of Evans Blue dye in the lateral ventricle.

2.3. Experiment 2: effects of central administration of GCGL on plasma glucose and corticosterone levels in chicks

Twenty eight 8-day-old layer chicks were weighed and allocated to two groups based on body weight (14 chicks in each group). GCGL (0 or 1 nmol) was intracerebroventricularly administered as described in Experiment 1. At 30 min after administration, chicks were sacrificed by decapitation, and their blood was collected. EDTA (1 mg/mL of blood) was used as the anticoagulant. Plasma was separated by centrifugation at 3000 × *g* for 10 min at 4 °C. Verification of the injection was made as described in Experiment 1. Plasma concentrations of glucose and corticosterone of the successfully administered chicks were measured using commercial kits (LabAssay™ Glucose, Wako Pure Chemical Industries, Ltd., Osaka, Japan; Correlate-EIA, Assay Designs, Inc., MI, USA).

2.4. Experiment 3: effects of co-administration of α-helical CRF on the CRF or GCGL-suppressed food intake in chicks

First, we examined the effect of central administration of α-helical CRF on food intake in layer chicks. Fourteen 8-day-old chicks were weighed and allocated to three groups based on body weight. After 2.5 h of fasting, chicks were administered either α-helical CRF (0, 0.26 or 1.3 nmol) into the lateral ventricle as described in Experiment 1. Then after 30 min of fasting, food was given, and food intake and verification of peptide administration was made as described in Experiment 1. We found that the central administration of 0.23 and 1.3 nmol α-helical CRF did not influence food intake (data not shown). Based on these results, doses 1.3 nmol was used in the following experiment.

Next, we examined the effect of central administration of α-helical CRF on the anorexigenic effect of CRF in chicks. Fifty four 8-day-old layer chicks were weighed and allocated to three groups based on body weight. After 2.5 h of fasting, chicks were administered α-helical CRF (0 or 1.3 nmol) into the right lateral ventricle. Then after 30 min of fasting, chicks were administered CRF (0 or 0.2 nmol) into the left lateral ventricle as described in Experiment 1. Subsequently, food was given, and food intake was measured at 30 min after administration. Verification of peptide administration was made by observation of the presence of Evans Blue dye in both right and left lateral ventricles.

Finally, we examined the effect of central administration of α-helical CRF on the anorexigenic effect of 1 nmol of GCGL in chicks as described above.

2.5. Experiment 4: effects of fasting on the mRNA levels of GCGL and GCGLR in chicken hypothalamus

Sixteen 8-day-old layer chicks were weighed and allocated to two groups based on body weight (8 birds in each group). Food was removed from 8 birds for 24 h, while the remaining 8 birds were allowed to feed freely. All birds had free access to water and were sacrificed by decapitation after 24 h. The hypothalamus was dissected from the brain by referring to a stereotaxic atlas [22] and stored at −80 °C for real-time PCR analysis. Total RNA was extracted from the hypothalamus using Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized using a ReverTra Ace® qPCR RT Master Mix with gDNA remover (TOYOBO CO. LTD., Osaka, Japan) with random primers. Complementary DNA of GCGL (GenBank accession no. EU718628) and GCGLR (GenBank accession no. EU718627) were amplified with the following primers: GCGL sense, 5′-AGA GCC CCA TCC AAC ACA AA-3′; GCGL antisense, 5′-GAG TGA AGT CGC TGG TGA AGG T-3′; GCGLR sense, 5′-ACC CCC GTG GTG TTT GTG-3′; GCGLR antisense, 5′-ACT CTG CGT TCT CCT TCA GGT ACT-3′. As an internal standard, complementary DNA of ribosomal protein S17 (RPS17) was also amplified with the primers as described previously [17]. mRNA expression was quantified in duplicate using the Applied Biosystems 7300 Real-Time PCR system and THUNDERBIRD™ SYBR® qPCR Mix (TOYOBO CO. LTD, Osaka, Japan) according to the supplier's recommendations.

2.6. Data analysis

Data from Experiments 1 and 3 were analyzed by the Tukey–Kramer test. Data from Experiments 2 and 4 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).