Glucagon-like Peptide-2 Functions as an Anorexigenic Peptide not only in the Central Nervous System but also in the Peripheral Circulation in Broiler Chicks

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Brain-gut peptides play important roles in the appetite regulatory system in mammals. Glucagon-like peptide (GLP)-1, GLP-2, and oxyntomodulin (OXM) are processed from the same precursor, proglucagon, in both brain and intestines in mammals and birds. We previously showed that intracerebroventricular administration of these three peptides significantly suppressed food intake in chicks. However, peripheral roles of chicken GLP-2 have not yet been investigated, although GLP-2 plays important roles both in central and peripheral regulation of food intake in mammals. The aim of this study is to investigate whether GLP-2 functions as an anorexigenic peptide in both brain and peripheral circulation in chicks. Intracerebroventricular administration of GLP-2 significantly suppressed food intake in chicks. Twenty-four hours of fasting significantly decreased the mRNA level of proglucagon in the medulla oblongata of chicks. These results suggest that GLP-2 functions as anorexigenic peptides in the central nervous system in chicks. In addition, intravenous administration of GLP-2 significantly suppressed food intake in chicks. Lines of evidence suggest that dietary nutrients stimulate the secretion of GLP-2 from L cells in the small intestine in chickens. These findings suggest that GLP-2 functions as both central and peripheral anorexigenic signals in chicks.

Key words: appetite, brainstem, chickens, hypothalamus, medulla oblongata

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Introduction

Broiler chickens, which are bred for rapid growth and high meat yield, do not adequately control voluntary food intake to meet their energy requirements (Richards and Proszkowiec-Weglarz, 2007). Consequently, their overconsumption of food can lead to excessive accumulation of visceral fat, which is regarded as an animal by-product or as waste. Thus, the regulatory mechanisms underlying food intake in poultry have been a focus of research in recent decades to improve production efficiency when raising chickens (Kuenzel 1994; Furuse, 2002; Bungo et al., 2011).

The proglucagon gene encodes three different peptides, such as glucagon-like peptide (GLP)-1, GLP-2, and oxyntomodulin (OXM), in both mammals (Kieffer and Habener, 1999) and birds (Richards and McMurtry, 2009). In response to food intake, these peptides are cosecreted from endocrine L cells in the gut and coreleased from the nucleus of solitary tract (NTS) in the brainstem in mammals (Guan, 2014). There is evidence that all of these peptides show anorexigenic effects in mammals (Turton et al., 1996; Tang-Christensen et al., 2000; Pocai, 2012). Thus, GLP-1, GLP-2, and OXM have been considered to act as appetite-regulating peptides in mammals.

Central administration of proglucagon-derived peptides such as OXM and GLP-1 suppresses food intake in chicks (Honda et al., 2014). In particular, the anorexigenic action of GLP-1 has been well investigated, and it has been demonstrated that GLP-1 acts as a potent anorexigenic peptide in the central nervous system in chicks (Furuse et al., 2007). We recently found that central administration of GLP-2 significantly suppressed food intake in layer chicks and suggested that, in addition to GLP-1, GLP-2 also acts as a potent anorexigenic peptide in the central nervous system at least in layer chicks (Honda et al., 2015). Lines of evidence have suggested that GLP-2 acts as one of the common hormones secreted by L cells in the small intestine in chickens. For example, GLP-2 colocalized with GLP-1 in the same secretory granules in chicken intestinal L cells.
(Monir et al., 2014b; Nishimura et al., 2013), and dietary protein and restriction feeding positively and negatively stimulated GLP-1-immunoreactive cells, respectively (Monir et al., 2014a, c). It is therefore possible that GLP-2 functioning as endocrine and neural signals may transmit intestinal nutrient sensing to the central nervous system to suppress food intake in chickens as well as in mammals. However, the physiological roles of GLP-2 in chickens have not been fully investigated.

In the present study, we focused on the central and peripheral effects of GLP-2 in broiler chicks. The results suggest that GLP-2 functions as both central and peripheral anorexigenic signals in chicks.

Materials and Methods

Animals and Peptides

Day-old male broiler chicks (Ross 308) and layer chicks (White Leghorn) were purchased from local hatcheries (Ishii Co. Ltd., Tokushima, Japan and Japan Layer K.K., Gifu, Japan, respectively). 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. 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 GLP-1 was purchased from Peptide Institute, Inc. (Osaka, Japan) as a custom peptide. Chicken GLP-2 was purchased from Funakoshi Co. Ltd. (Tokyo, Japan) as a custom peptide. Chicken OXM was purchased from Biologica Co. (Nagoya, Japan) as a custom peptide.

Experiment 1: Effect of Central Administration of GLP-2 on Food Intake in Chicks

Fifty six 8-day-old broiler chicks were weighed and allocated to four groups based on body weight (14 birds in each group). Chicken GLP-2 was dissolved in a saline solution containing 0.1% (w/v) Evans Blue. The peptide (0, 30, 100, or 300 pmol) was intracerebroventricularly administered according to the method of Davis et al., (1979) at a volume of 10μL 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 sacrificed by decapitation. Verification of injection was made by observation of the presence of Evans Blue dye in the lateral ventricle.

Experiment 2: Effect of Central Administration of Proglucagon-derived Peptides on Food Intake in Chicks

Fifty five 4-day-old broiler chicks were weighed and allocated to four groups based on body weight (11 birds in each group). Either 10 pmol of GLP-1, GLP-2, or OXM was intracerebroventricularly administered as described above. Food intake was measured at 60, 120, and 180 min after administration. At the end of the experiment, the chicks were sacrificed by decapitation. Verification of injection was made as described above.

Experiment 3: Effect of Fasting on the mRNA Level of Proglucagon in the Medulla Oblongata in Chicks

Twelve 7-day-old broiler chicks were weighed and allocated to two groups, feeding and fasting groups, based on body weight (six birds in each group). After 24 h of ad libitum feeding or fasting, chicks were sacrificed by decapitation and the medulla oblongata was excised, immediately frozen by liquid nitrogen and stored at −80°C. Total RNA was extracted from the frozen medulla oblongata using the Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand complementary DNA (cDNA) was synthesized from total RNA using a ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co. Ltd., Osaka, Japan). Complementary DNAs of proglucagon (GenBank accession no. Y07539) and ribosomal protein S17 (RPS17, GenBank accession no. NM_204217) were amplified with the following primers: proglucagon sense, 5'- CGA GAG TTC ATT ACG TTA AAG GTT-3'; antisense, 5'- TGT AGG TGC CTT CAG CAT GTC T-3'; RPS17 sense, 5'-GCG GGT GAT CAT CGA GAA GT-3'; antisense, 5'-GCC CTT GTT GGT GTG GAA GT-3'. All primers were purchased from Hokkaido System Science Co., Ltd. (Hokkaido, Japan). THUNDERBIRD™ SYBR® qPCR Mix was purchased from Toyobo Co. Ltd. (Osaka, Japan), and mRNA expression was quantified in duplicate using the Applied Biosystems 7300 Real-Time PCR system according to the supplier’s recommendations.

Experiment 4: Effect of Peripheral Administration of GLP-2 on Food Intake in Chicks

Twenty 8-day-old broiler chicks were weighed and allocated to two groups based on body weight (10 birds in each group). GLP-2 was dissolved in a saline solution. The peptide (0 or 1.5 nmol/2 ml/kg body weight) was administered via a wing vein under ad libitum feeding condition. Food intake was measured at 30 and 60 min after administration.

Statistical Analysis

Data from Experiment 1 and 2 were analyzed by the Tukey-Kramer test. Data from Experiment 3 and 4 were analyzed by Student’s t-test. All statistical analyses were performed using the commercial package (StatView version 5, SAS Institute, Cary, NC, USA, 1998).

Results

In the present study, we first examined the effect of central administration of GLP-2 on food intake in chicks. Intracerebroventricular administration of GLP-2 significantly (p <0.05) suppressed food intake (Fig. 1). We also compared the effects of GLP-1, GLP-2, and OXM on food intake at a low dose (10 pmol) because these three peptides were co-produced in the proglucagon-expressing neurons in the NTS. Central administration of GLP-1, GLP-2, but not OXM, significantly suppressed food intake (Fig. 2). The anorexigenic effects of GLP-1 and GLP-2 were abolished at 3 h after injection (Fig. 2).

We next examined the effect of fasting on proglucagon expression in the medulla oblongata of chicks in order to evaluate the role of endogenous proglucagon-derived peptides. Twenty-four hours of fasting significantly decreased the mRNA level of proglucagon (Fig. 3).
We finally examined the effect of the peripheral administration of GLP-2 on food intake in chicks and found that its intravenous administration of GLP-2 signif icantly decreased food intake (Fig. 4).

Discussion

In mammals, GLP-2 is degraded by the enzyme dipeptidyl peptidase-IV, which renders the peptide inactive by N-terminal truncation of the alanine at position 2 in plasma (Drucker et al., 1997). There is evidence of dipeptidyl peptidase IV in the brain in mammals (Deacon, 2004). Accurate assessment of GLP-2 degradation products in the central nervous system in chickens has yet to be performed. However, in the present study, the anorexigenic effect of GLP-2 was observed at least 2 hours after central administration (Figs. 1 and 2). Thus, GLP-2 might be a relatively long-acting peptide in the central nervous system in chicks.

Central administration of a low dose (10 pmol) of GLP-1 and GLP-2, but not OXM, significantly suppressed food intake (Fig. 2), suggesting that, in addition to GLP-1, GLP-2 acts as a potent anorexigenic peptide in the central nervous system in chicks. In mammals, GLP-1 and GLP-2 exhibit biological actions mediated by different receptors (Mayo et
In chickens, GLP-1 and GLP-2 receptor genes are expressed in the brain (Richards and McMurtry, 2008). Recently, Mo et al. (2014) demonstrated that chicken GLP-2 receptor expressed in CHO cells could be potently activated by chicken GLP-2 but not by other structurally related peptides, including chicken GLP-1 and glucagon, suggesting that GLP-2 receptor is a functional receptor specific to GLP-2 in chickens as well as in mammals. In contrast, chicken GLP-1 receptor expressed in Chinese hamster ovary (CHO) cells could not be activated by GLP-2 (Huang et al., 2012). These findings suggest that GLP-2 express their functions via its own receptor.

The expression of appetite regulatory neuropeptides is known to be changed by fasting or food deprivation in chickens. For example, the mRNA level of an orexigenic peptide, neuropeptide Y, in the hypothalamus was increased by fasting in chicks (Kameda et al., 2001), whereas the mRNA level of the precursor of anorexigenic peptides, proopiomelanocortin, in the hypothalamus was reduced by restriction feeding in chicks (Hen et al., 2006). In mammals, it is assumed that all axonal terminal fields in the central nervous system containing proglucagon-derived peptides originate in the NTS in the medulla oblongata (Vrang and Larsen, 2010). In the present study, 24 h of fasting significantly decreased the mRNA level of proglucagon in the medulla oblongata of broiler chicks (Fig. 3). This result is in good agreement with the results in layer chicks (Tachibana et al., 2005). These findings suggest that GLP-1 and GLP-2 act as endogenous anorexigenic neuropeptides in both broiler and layer chicks.

To the best of our knowledge, in birds, there is only one report showing no effect of the peripheral administration of rat GLP-2 on food intake in Japanese quails (Shousha et al., 2007). However, rat GLP-2 shares only 52% amino acid identity with chicken GLP-2 (Honda et al., 2015). In fact, rat GLP-2 did not influence food intake in Japanese quails when administered centrally (Shousha et al., 2007). Thus, we finally examined the effect of peripheral administration of chicken GLP-2 on food intake in chicks and found that its intravenous administration significantly decreased food intake in chicks (Fig. 4). Richards and McMurtry (2013) reported that fasting had no effect on proglucagon mRNA levels in the duodenum of chickens. However, restriction feeding significantly increased GLP-1-immunoreactive cells in the chicken small intestine (Monir et al., 2014c). Multiple regression analysis indicated a significant correlation between the daily protein intake and frequencies of occurrence of GLP-1-immunoreactive cells (Monir et al., 2014a). GLP-2 colocalized with GLP-1 in the same secretory granules in the ileum (Nishimura et al., 2013). Monir et al. (2014b) suggest that GLP-2 may act as one of the common hormones secreted by L cells in the chicken small intestine. These findings and our results suggest that GLP-2 functions as an anorexigenic peptide not only in the central nervous system but also in the peripheral circulation in chicks.

Modern broiler chickens eat more food than layer chicks (Saneyasu et al., 2011), and their overconsumption of food can lead to excessive accumulation of visceral fat (Wang et al., 2010). Thus, extensive selective breeding of chickens for high meat yield has resulted in obese phenotype. In mammals, diet-induced obese mice are less sensitive to the anorectic effect of GLP-2 when administered peripherally (Baldassano et al., 2012). However, in the present study, GLP-2 significantly suppressed food intake in broiler chicks, although the dose (about 5.7 ng/g body weight) was lower than that in mammalian study (900 ng/g body weight) (Baldassano et al., 2012). It is therefore possible that GLP-2 functions as a potent anorexigenic peptide even though in broiler chickens when compared with that in mammals.

In mammals, peripheral anorexigenic action of GLP-2 is suggested to be mediated via endocrine and neural manners. Guan (2014) proposed that peripheral GLP-2 can be transported into the hypothalamic arcuate nucleus, where the blood-brain barrier is semipermeable or taken up through the median eminence. Nelson et al. (2007) showed that peripheral administration of GLP-2 stimulates neural activity in the central nervous system in a manner dependent on vagal afferents. In chickens, GLP-2 receptor was highly expressed in pancreas, brain, and gastrointestinal tracts, suggesting that GLP-2 effect associate with these tissues (Richards and McMurtry, 2008). It is therefore possible that the anorexigenic action of peripherally administered GLP-2 is due to the direct effect of circulating GLP-2 and/or indirect neurally mediated effects via peripheral GLP-2 receptors. Further studies will be needed to clarify these points.

In conclusion, intracerebroventricular administration of GLP-2 significantly decreased food intake in chicks. The mRNA levels of proglucagon in the medulla oblongata were significantly decreased by fasting. In addition, intravenous administration of GLP-2 significantly decreased food intake in chicks. These findings suggest that GLP-2 functions as an anorexigenic peptide not only in the central nervous system but also in the peripheral circulation in chicks.

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