Comparative Biochemistry and Physiology, Part A 159 (2011) 422–426

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Neuropeptide Y effect on food intake in broiler and layer chicks

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ARTICLE INFO

Article history:
Received 4 March 2011
Received in revised form 14 April 2011
Accepted 15 April 2011
Available online 29 April 2011

Keywords:
Appetite
Avian
Feeding behavior
NPY
POMC

ABSTRACT

Broiler chicks eat more food than layer chicks. In this study, we examined the involvement of orexigenic peptide neuropeptide Y (NPY) in the difference in food intake between broiler and layer chicks (*Gallus gallus*). First, we compared the hypothalamic mRNA levels of NPY and its receptors (Y1 and Y5 receptors) between these strains at 1, 2, 4, and 8 days of age. Daily food intake was significantly higher in broiler chicks than layer chicks after 2 days of age. However, the hypothalamic NPY mRNA level was significantly lower in broiler chicks than layer chicks except at 8 days of age. In addition, the mRNA levels of NPY receptors were also significantly lower in broiler chicks than layer chicks at 2 and 4 days of age (Y1 receptor) or 2 days of age (Y5 receptor). These results suggest that the differences in the expressions of hypothalamic NPY and its receptors do not cause the increase in food intake in broiler chicks. To compare the orexigenic effect of NPY between broiler and layer chicks, we next examined the effects of central administration of NPY on food intake in these strains. In both strains, central administration of NPY significantly increased food intake at 2, 4 and 8 days of age. All our findings demonstrated that the increase in food intake in broiler chicks is not accompanied with the over-expression of NPY or its receptor.

1. Introduction

Broiler chickens have been genetically selected for their heavy body weight and high growth rate, and genetic selection has introduced wide variations in food intake between broiler and layer chickens. There is evidence showing a significant increase in food intake in broiler chickens compared to layer chickens from the neonatal period (Nir et al., 1993; Mahagna and Nir, 1996). However, little is known the detailed mechanism involved in this increase in food intake in broiler chickens.

In mammals, neuropeptide Y (NPY) and agouti-related protein (AgRP) play an important role in the stimulation of food intake in the hypothalamus (Morton et al., 2006). NPY and AgRP were highly expressed in the hypothalamic arcuate nucleus (Morris, 1989; Morton et al., 2006). The mRNA levels of NPY and AgRP in the hypothalamus are increased by fasting (Bertile et al., 2003) and reduced by peripheral anorexigenic hormone insulin (Schwartz et al., 1992; Qu et al., 2001) and leptin (Stephen et al., 1995; Shutter et al., 1997). In fact, the hyperphagia of genetically obese animals such as leptin-deficient mice and leptin receptor-deficient rats is induced by an increase in hypothalamic NPY and AgRP mRNA levels (McKibbin et al., 1991; Wilding et al., 1993; Shutter et al., 1997). The orexigenic effect of NPY is mediated by hypothalamic Y1 and Y5 receptors (Gerald et al., 1996; Kanatani et al., 2000). Both the Y1 receptor antagonist and Y5 receptor antagonist significantly reversed NPY-induced food intake (Kakui et al., 2006;Kanatani et al., 2001). On the other hand, AgRP antagonizes anorexigenic neuropeptide alpha-melanocyte stimulating hormone (α-MSH) binding and signaling via the melanocortin 4 receptor (Nijenhuis et al., 2001).

In birds, AgRP increases food intake in layer chicks but not in broiler chicks, suggesting that AgRP is not a cause of the increase in food intake in broiler chicks (Tachibana et al., 2001). In contrast, central administration of NPY significantly increases food intake in broiler (Kuenzel et al., 1987; Kawakami et al., 2000; Ando et al., 2001; Cline and Smith, 2007) and layer chicks (Saito et al., 2005; Tachibana et al., 2006), as well as in mammals. NPY mRNA was highly expressed in the infundibular nucleus, the avian equivalent of the mammalian arcuate nucleus (Kameda et al., 2001; Wang et al., 2001), and the mRNA level of NPY in the hypothalamus is increased by fasting (Kameda et al., 2001) and restriction feeding (Boswell et al., 1999) and reduced by insulin (Shiraishi et al., 2008) and leptin (Dridi et al., 2005). NPY-immunoreactive fibers were seen throughout the entire chick brain, but were more abundant in the hypothalamus (Esposito et al., 2001). NPY content in the paraventricular nucleus and infundibular nucleus significantly increased during fasting, and NPY content of the PVN was restored to pre-fasting levels after 24-h refeeding (Zhou et al., 2005). NPY and its receptors (Y1, Y2, Y4, Y5, Y6, and Y7) have been cloned (Blomqvist et al., 1992; Salaneck et al.,

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doi:10.1016/j.cbpa.2011.04.008

2000; Holmberg et al., 2002; Lundell et al., 2002; Bromée et al., 2006), and Y1 and Y5 receptors are expressed in the chicken hypothalamus (Holmberg et al., 2002). Thus, NPY plays a critical role in the stimulation of food intake in chicks.

In the present study, to clarify the involvement of NPY in the difference in food intake between broiler and layer chickens, we compared the hypothalamic mRNA levels of NPY, the Y1 receptor, and Y5 receptor, and the orexigenic effect of NPY between broiler and layer chicks at 1, 2, 4, and 8 days of age.

2. Materials and methods

2.1. Animals

Day-old male chicks (*Gallus gallus*) of a broiler (chunky: Ishii Co., Ltd. Tokushima, Japan) and a layer (White Leghorn: Ghen Corporation, Gifu, Japan) were purchased from a local hatchery. 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 with an automatically controlled 12-h light:dark cycle (6:00–18:00). All experimental procedures followed the guidelines for the care and use of experimental animals at the Rokkodai Campus of Kobe University in Japan. Chicken NPY was purchased from the Peptide Institute, Inc. (Osaka, Japan).

2.2. Experiment 1: daily food intake of broiler and layer chicks

In Experiment 1, at 1 day of age, eight chicks of each strain were housed in individual cages with free access to water and a commercial diet. Food intake was measured every day.

2.3. Experiment 2: changes in the hypothalamic neuropeptide Y mRNA level in broiler and layer chicks

Broiler and layer chicks were sacrificed at the mid-point of the light phase (12:00) by decapitation at 1, 2, 4, and 8 days of age. Their brains were removed within 1 min of decapitation, weighed, frozen on powdered dry ice and stored at −80 °C for further analysis. The hypothalamus was dissected from the frozen brain, by referring to a stereotaxic atlas (Kuenzel and Masson, 1988), and was weighed. Total RNA was extracted from the hypothalamus using Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from 5 μg of DNase I (Ambion Inc., Austin, Texas, USA)-treated total RNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems Inc., Foster city, California, USA) with random primers. Complementary DNA of NPY (GenBank accession no. **NM_205473**) was amplified with the following primers: NPY sense, 5′-CTT GTC GCT GCT GAT CTG -3′; NPY antisense, 5′-GCC TCA GAG CCG AGT AGT-3′. As an internal standard, chicken ribosomal protein S17 mRNA (**NM_204217**) was also amplified, using the following primers: sense, 5′-GCG GGT GAT CAT CGA GAA GT-3′; antisense, 5′-GCG CTT GTT GGT GTG GAA GT-3′. SYBR® Premix Ex Taq was purchased from Takara Bio Inc. (Shiga, Japan), and mRNA expression was quantified in triplicate using the Applied Biosystems 7300 Real-Time PCR system according to the supplier's recommendations. After the reactions, the specificity of amplifications in each sample was confirmed by dissociation analysis showing that each sample gave a single melting peak. Relative gene expression was calculated by comparing the number of thermal cycles that were necessary to generate threshold amounts of product (CT). CT was calculated for the NPY and for the chicken ribosomal protein S17. For each cDNA sample, the CT for chicken ribosomal protein S17 was subtracted from the CT for NPY to give the parameter ΔCT, thus normalizing the initial amount of RNA used. The amount of NPY mRNA was calculated as $2^{-\Delta\Delta CT}$, where ΔΔCT is the difference between the ΔCT of the two cDNA samples to be compared.

2.4. Experiment 3: changes in hypothalamic mRNA levels of Y1 and Y5 receptors in broiler and layer chicks

Hypothalamic mRNA levels of Y1 and Y5 receptors were analyzed as described in Experiment 2. Complementary DNAs of Y1 receptor (GenBank accession no. **NM_001031535**) and Y5 receptor (**NM_001031130**) were amplified with the following primers: Y1 receptor sense, 5′-TAG CCA TGT CCA CCA TGC A-3′; Y1 receptor antisense, 5′-GGG CTT GCC TGC TTT AGA GA-3′; Y5 receptor sense, 5′-GGC TGG CTT TGT GGG AAA-3′; and Y5 receptor antisense, 5′-TTG TCT TCT GCT TGC GTT TTG T-3′.

2.5. Experiment 4: effect of central administration of neuropeptide Y on cumulative food intake in broiler and layer chicks

Broiler and layer chicks were divided into three groups at 1, 2, 4, and 8 days of age. Chicken NPY (YPSKPDSPGEDAPAEDMARYYSALRHYINLITRQRY-NH2) was purchased from Peptide Institute, Inc. (Osaka, Japan). NPY was dissolved in a 0.85% (w/v) saline solution containing 0.1% (w/v) Evans Blue. Either NPY (0.2, or 0.4 μg) or saline (as a control) was intracerebroventricularly administered according to the method of Davis et al. (1979) 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 sacrificed by decapitation. Verification of injection was made by observation of the presence of Evans Blue dye in the lateral ventricle.

2.6. Data analysis

Data from Experiments 1, 2, and 3 were analyzed by Student's *t* test at each day of age. Data from Experiment 4 were analyzed by the Tukey-Kramer test at each time point. All statistics was performed using a commercial software package (StatView version 5, SAS Institute, Cary, NC, USA, 1998).

3. Results and discussion

First, we compared the difference in food intake between broiler and layer chicks. The daily food intake of broiler chicks was significantly higher than that of layer chicks after 2 days of age (Fig. 1).

We next examined the hypothalamic NPY mRNA level in broiler and layer chicks. The hypothalamic NPY mRNA level was significantly lower in broiler chicks than layer chicks at 1, 2, and 4 days of age

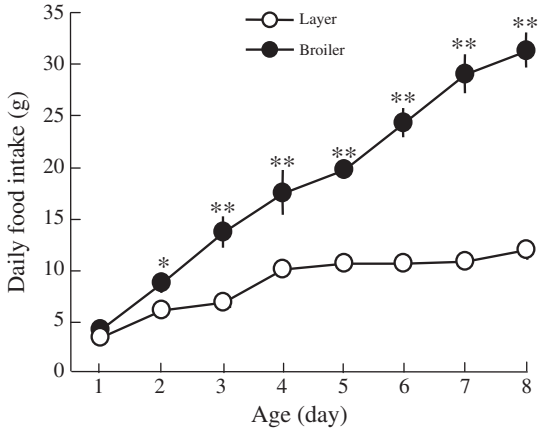

Fig. 1. Daily food intake of broiler and layer chicks. Data are means ± S.E.M. of eight chicks in each group. * and **, significant with respect to layer chicks ($p<0.05$ and $p<0.01$).