Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 150 (2008) 21–25
CBP
www.elsevier.com/locate/cbpa

The orexigenic effect of GnIH is mediated by central opioid receptors in chicks

Tetsuya Tachibana [a,*], Naoto Masuda [a], Kazuyoshi Tsutsui [b], Kazuyoshi Ukena [c], Hiroshi Ueda [a]

[a] *Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama, Ehime 790-8566, Japan*
[b] *Laboratory of Integrative Brain Sciences, Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, Tokyo 169-8050, Japan*
[c] *Laboratory of Brain Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739-8521, Japan*

Received 9 January 2008; received in revised form 9 February 2008; accepted 15 February 2008
Available online 23 February 2008

Abstract

Gonadotropin-inhibiting hormone (GnIH) is a newly discovered hypothalamic hormone which suppresses gonadotropin synthesis and release from the anterior pituitary. Recently, we found that intracerebroventricular (ICV) injection of GnIH stimulated feeding behavior of chicks (*Gallus gallus*) and suggested that GnIH is one of orexigenic peptides. However, the mechanism underlying the orexigenic effect is still unknown. In the present study, we examined whether the orexigenic effect of GnIH is related to opioid and nitric oxide (NO) systems. The orexigenic effect of ICV-injected GnIH was attenuated by co-injection of beta-funaltrexamine (an opioid mu-receptor antagonist) but not ICI-174,864 and nor-binaltorphimine (antagonists of opioid delta- and kappa-receptors, respectively). The co-injection of non-selective NO synthase inhibitor did not affect GnIH-induced feeding behavior. The present study demonstrated that the GnIH-induced feeding might be mediated by opioid mu-receptor in chicks.

Keywords: Chicks; Feeding; Gonadotropin-inhibiting hormone; Intracerebroventricular injection; Opioid

1. Introduction

Gonadotropin-inhibiting hormone (GnIH) is a newly discovered hypothalamic hormone which inhibits gonadotropin release from the quail anterior pituitary (Tsutsui et al., 2000). In addition to the inhibitory effects, it is expected that GnIH has several other physiological actions in the brain since GnIH containing-neurons (Ukena et al., 2003) and GnIH receptor (Yin et al., 2005) are widely distributed in the avian brain. We previously reported that intracerebroventricular (ICV) injection of GnIH stimulates feeding behavior of 6-day-old layer chicks (Tachibana et al., 2005), suggesting that GnIH is one of orexigenic peptides in the brain. On the other hand, ICV injection of GnIH did not affect testosterone release in 6-day-old male layer chicks (Tachibana et al., 2005). Additionally, the GnIH-induced feeding was confirmed in immature neonatal chicks, demonstrating that the gonad might not be related to the effect. Therefore, the mechanism underlying the orexigenic effect is still unknown.

In chicks, it is reported that some of orexigenic factors found in mammals do not stimulate feeding behavior. For example, ICV injection of motilin, melanin-concentrating hormone, orexin, galanin, ghrelin and growth hormone-releasing hormone did not stimulate feeding behavior of chicks (Furuse et al., 1999; Ando et al., 2000; Furuse et al., 2001). Among them, neuropeptide Y (NPY) and opioid peptides are thought to stimulate feeding behavior in chicks. Several previous studies revealed that ICV injection of NPY stimulated feeding behavior of chicks (Kuenzel et al., 1987; Tachibana et al., 2004; Dodo et al., 2005). Similar to this, ICV injection of opioids such as beta-endorphin (McCormack and Denbow, 1988), methionine-enkephalin (McCormack and Denbow, 1989) and endomorphin-2 (Bungo et al., 2007) stimulated feeding behavior of chicks while dynorphin did not (Steinman et al., 1987). Nitric oxide (NO) is also thought to be related to stimulate feeding behavior because ICV injection of NO synthase (NOS) inhibitor

* Corresponding author. Tel./fax: +81 89 946 9820.
E-mail address: tetsu@agr.ehime-u.ac.jp (T. Tachibana).

1095-6433/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2008.02.018

decreased food intake of chickens (Choi et al., 1994). It was reported that the brain opioid and NO mediated the orexigenic effect of NPY in chickens (Bungo et al., 2000; Dodo et al., 2005). This suggested that these factors might play as the mediator in the orexigenic mechanism in the brain of chicks.

GnIH-containing perikarya, nerve fibers and GnIH are found in the hypothalamus of quails (Ukena et al., 2003; Yin et al., 2005). On the other hand, several previous studies revealed that opioid-containing neurons such as methionine-enkephalin (De Lanerolle et al., 1981) and beta-endorphin (Van Gils et al., 1994) are found in the hypothalamus of chickens. NADPH-diaphorase, an enzyme catalyzed reaction thought to reflect the activity of NOS was also observed in the hypothalamus of chickens (Bruning, 1993). It is possible that GnIH-induced feeding might be related to the brain opioid and NO systems.

The purpose of the present study was to clarify the mechanism underlying the orexigenic effect of GnIH. First, we investigated whether GnIH stimulates feeding behavior of broiler chicks which eat more diet and grow faster than layer chicks (National Research Council, 1994). Second, whether opioid and NO systems are involved in the orexigenic effect of GnIH was examined.

2. Materials and methods

2.1. Animals

Day-old male broiler chicks (*Gallus gallus*, Chunky, purchased from Mori Hatchery, Kagawa, Japan) were raised in a room kept at 30 °C with continuous lighting. Commercial diet (Toyohashi Feed Mills Co. Ltd, Aichi, Japan) and water were freely given to chicks. Before each experiment, body weight was measured and the chicks were distributed into experimental groups so that the average body weight was as uniform as possible between groups in each experiment. Chicks were maintained in accordance with the recommendations of the National Research Council (National Research Council, 1996).

2.2. Drugs and ICV injection

Synthetic quail GnIH (Tsutsui et al., 2000) was used in the present study. Beta-funaltrexamine (FNA), ICI-174,864 (ICI) and nor-binaltorphimine (BNI), which are antagonists of opioid mu-, delta- and kappa-receptors, respectively, were purchased from Sigma Aldrich, St. Louis, USA. N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective NOS inhibitor, was also purchased from Sigma Aldrich. Drugs were dissolved in a saline solution containing 0.1% Evans Blue solution. This saline solution was used for the control treatment.

The ICV injection was performed according to a method reported previously (Davis et al., 1979). Briefly, the head of a chick was inserted into an acrylic box which had a hole at the top plate. A microsyringe was then inserted into the hole and drug solution was injected at a volume of 10 μL. This method had been defined as unstressful based on the previous studies (Furuse et al., 1999; Saito et al., 2005). The ICV injection was done under an *ad libitum* feeding condition.

At the end of each experiment, chicks were sacrificed with an overdose of pentobarbital. Their brains were then removed for the confirmation of drug injection. Chicks which did not show Evans Blue dye in the ventricle were not used for data collection.

2.3. Experiment 1: effect of ICV injection of GnIH on feeding behavior of broiler chicks

Three-day-old broiler chicks were ICV injected with 0 nmol (control), 0.9 nmol or 2.6 nmol GnIH. The doses of GnIH were decided according to our previous study (Tachibana et al., 2005). Food intake was measured at 30 and 60 min after the injection. The number of chicks was as follows: 0 nmol (control), 7; 0.9 nmol, 6; 2.6 nmol, 8.

2.4. Experiment 2: effect of opioid receptor antagonist on GnIH-induced feeding behavior

FNA, ICI and BNI were ICV co-injected with GnIH to examine whether the GnIH-induced feeding were mediated by opioid mu-, delta- and kappa-receptors, respectively. The dose of GnIH was fixed at 2.6 nmol based on the previous study (Tachibana et al., 2005). The doses opioid receptor antagonists were decided according to the previous reports (Dodo et al., 2005). The food intake was measured at 30 min after the injection.

The mu-receptor study was performed using 4-day-old chicks and they were ICV injected with saline (control), GnIH, 20 nmol FNA or GnIH plus 20 nmol FNA. The number of chicks was as follows: control, 8; GnIH, 8; FNA, 9; GnIH plus FNA, 8.

In the delta-receptor study, 2-day-old chicks were ICV injected with saline (control), GnIH, 6 nmol ICI or GnIH plus 6 nmol ICI. The number of chicks was as follows: control, 7; GnIH, 8; ICI, 8; GnIH plus ICI, 9.

The kappa-receptor study was done using 4-day-old chicks and they were ICV injected with saline (control), GnIH, 2.7 nmol BNI or GnIH plus 2.7 nmol BNI. The number of chicks was as follows: control, 8; GnIH, 9; BNI, 7; GnIH plus BNI, 7.

2.5. Experiment 3: effect of NOS inhibitor on GnIH-induced feeding behavior

L-NAME was ICV co-injected with GnIH to investigate whether the orexigenic effect of GnIH is related to NO system. In this experiment, 3-day-old chicks were injected with saline (control), 2.6 nmol GnIH, 400 nmol L-NAME or GnIH plus L-NAME. The dose of L-NAME was decided according to the previous study (Tomonaga et al., 2005). The number of chicks in each group was as follows: control, 8; GnIH, 6; L-NAME, 6; GnIH plus L-NAME, 7.

2.6. Statistical analysis

Data of Experiment 1 were statistically analyzed with two-way repeated measures analysis of variance (ANOVA). Data of Experiments 2–4 were analyzed by two-way ANOVA with respect to GnIH and each antagonist or inhibitor. The Tukey–