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Feeding responses to μ-, δ- and κ-opioid receptor agonists in the meat-type chick

Takashi Bungo*, Kazuya Kawamura, Tomofumi Izumi, Koh-Ichi Dodo, Hiroshi Ueda

Laboratory of Animal Science, Department of Agrobiological Science, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan

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Abstract

The present study was designed to examine the effect of specific opioid agonists on feeding behavior in neonatal chicks. The food intake of broiler chicks was significantly decreased by intracerebroventricular injection of DAMGO and β-casomorphin, μ-opioid receptor agonists, at 30-min postinjection. In contrast, both δ-opioid receptor agonists (DADLE and DPDPE) stimulated the food intake of the chick. Similar to the δ-opioid receptor agonists, food intake was elevated by the κ-opioid receptor agonist (U-50488H and U-62066) in a dose-dependent manner. These results suggest that the endogenous opioid peptides have an important role for feeding behavior in the central nervous system of chicks.

Keywords: μ Receptor; δ Receptor; κ Receptor; Food intake; Intracerebroventricular injection; Chick

1. Introduction

Opioids are known to be inhibitory neurotransmitters, and their receptors, classified mainly as μ, δ or κ subtypes, are widely distributed throughout the central nervous system in vertebrates (Akil et al., 1984). These receptors and their ligands have been shown to be important in a variety of functions, such as analgesia (Kanjhan, 1995), recognition (D'amato, 1997) and cardiorespiratory control (Zhang and Moss, 1995). In addition, several reports have indicated the endogenous opioid system in the modulation of feeding behavior in mammals (e.g., Bodnar, 1996).

Some opioid peptides that are present in the brain are the same in birds and mammals (Kotegawa et al., 1995), and high concentrations of Met-enkephalin are found in the avian neurohypophysis (Martin et al., 1992). Additionally, autoradiographic studies indicate that the chick brain contains μ-, δ-, and κ-opioid receptor subtypes (Csillag et al., 1990). Therefore, similar to mammals, these observations suggest that endogenous opioid peptides may represent important signals in the regulation of the feeding behavior of chickens. In fact, the central injection of Met-enkephalin and β-endorphin induced hyperphagia in chickens (McCormack and Denbow, 1988, 1989; Savory et al., 1989). Moreover, dynorphin suppressed food consumption of layer-type chicks (Steinman et al., 1987).

Because chickens, as a precocial species, recognize and ingest food voluntarily, it is important to investigate the feeding behavior of neonatal chicks. However, the effects of specific opioid agonists on the feeding behavior of meat-type chicks have not been studied. Thus, the purpose of the present experiment was to determine if specific opioid agonists act within the central nervous system of neonatal chicks to control ingestive behavior.

2. Materials and methods

Day-old male broiler chicks (Cobb) were purchased from a local hatchery (Fresh Foods, Ehime, Japan). The birds were maintained in a room with 24-h lighting and a temperature of 30 °C. They were given free access to a commercial starter diet (Nihon Nosan Kogyo, Yokohama, Japan) and water during the preexperimental period. The chicks were maintained in accordance with the recommendations of the National Research Council

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

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(1985). They were distributed into experimental groups based on their body weight so that the average body weight was as uniform as possible for each treatment. The birds were reared individually in experimental cages and had ad libitum access to food up to the time of experiments. The birds (2- or 3-day old) fed ad libitum were given diet for 2 h immediately after treatment. Food intake was determined by measuring the reduction of diet from a preweighed feeder. The weight of the feeders was measured using an electric digital balance of precision ± 1 mg.

[D-Ala^2, *N*-MePhe^4, Gly^5-ol]-enkephalin (DAMGO), a selective μ-opioid receptor agonist (Minami and Satoh, 1995), [D-Ala^2, D-Leu^3]-enkephalin (DADLE), a preferential δ-opioid receptor agonist (Minami and Satoh, 1995); [D-Pen^{2,5}]-enkephalin (DPDPE), a selective δ-opioid receptor agonist (Minami and Satoh, 1995); U-50488H, a selective κ-opioid receptor agonist (Minami and Satoh, 1995); and U-62066, a highly selective κ-opioid receptor agonist (Holtzman, 2000) were purchased from Sigma (St. Louis, MO, USA), and β-casomorphin (bovine), a preferential μ-opioid receptor agonist (Koch et al., 1985) was obtained from Peptide Inc. (Osaka Japan). The drugs were dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. Saline containing Evans Blue was used as the control. The birds were intracerebroventricularly injected with the solutions (10 μl) using a microsyringe according to the methods used by Davis et al. (1979). Each chick was injected once only with a dose of either agonist or saline. The doses applied here were decided according to the reports of mammals (Gosnell and Levine, 1996) and chickens (McCormack and Denbow, 1989).

At the end of the experiments, the birds were sacrificed by decapitation, after which the location of the injection site was confirmed. Data from the individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were deleted. The number of birds used for data analysis is shown in each figure.

The data were subjected to one-way and repeated-measure two-way analysis of variance (ANOVA) using a commercially available package (Stat View, Version 5, SAS Institute, Cary, NC). Significant differences of food intake of chicks at each time were detected using Fisher's PLSD test ($P<.05$). The results are presented as means ± SEM.

3. Results

Fig. 1 shows the effect of intracerebroventricular injection of μ-opioid receptor agonists on food intake in chicks. The effect of DAMGO was significant for food intake in full-fed chicks [Fig. 1A; $F(3,25)=3.903$, $P<.05$]. The effect of β-casomorphin was also significant for food intake [Fig. 1B; $F(3,21)=3.726$, $P<.05$]. A

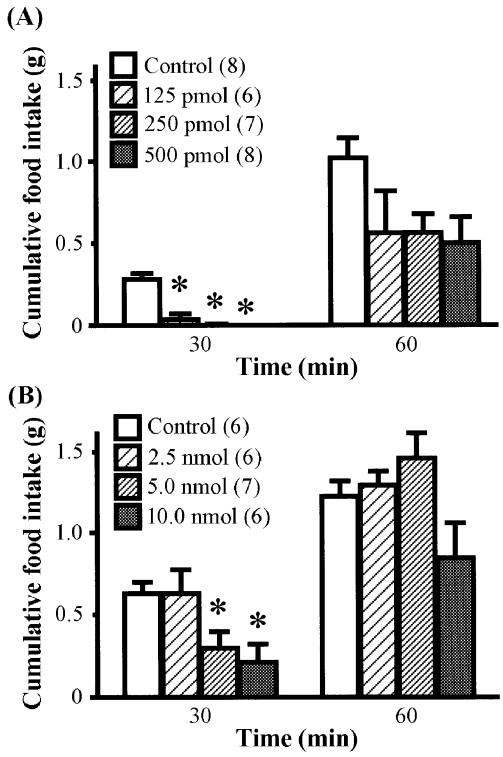

Fig. 1. Effects of intracerebroventricular injection of μ-opioid receptor agonists (A: DAMGO; B: β-casomorphin) on food intake in chicks. Values are means ± S.E.M. of the number of chicks (shown in parentheses). * $P<.05$, compared with saline control.

significant interaction was detected between time and β-casomorphin [Fig. 1B; $F(6,42)=3.110$, $P<.05$], but not DAMGO. Food intake at 30 min was suppressed by the intracerebroventricular injection of both μ-opioid receptor agonists, but no significant effects were observed at 60 min.

The effects of the intracerebroventricular injection of δ-opioid receptor agonists on food intake in chicks are shown in Fig. 2. Although food intake was enhanced by the intracerebroventricular administration of DADLE at 60 min [Fig. 2A; $F(3,29)=4.127$, $P<.05$], a significant interaction was not detected between time and DADLE. The effect of DPDPE was also significant for food intake in chicks [Fig. 2B; $F(3,33)=4.821$, $P<.05$]. A significant interaction was detected between time and DPDPE [$F(6,66)=2.695$, $P<.05$]. All doses of DPDPE elicited food consumption of chicks at 30 min, but no significant effects were observed at 60 min.

Fig. 3 shows the effects of the intracerebroventricular injection of κ-opioid receptor agonists (U-50488H and U-62066) on food intake in chicks. Food intake at 30 min was increased by the intracerebroventricular injection of each κ-opioid receptor agonist [Fig. 3A: $F(2,47)=7.774$, $P<.05$; Fig. 3B: $F(2,30)=3.431$, $P<.05$], but no significant effects were observed at 60 min. A significant interaction between time and each κ-opioid receptor agonist was also detected

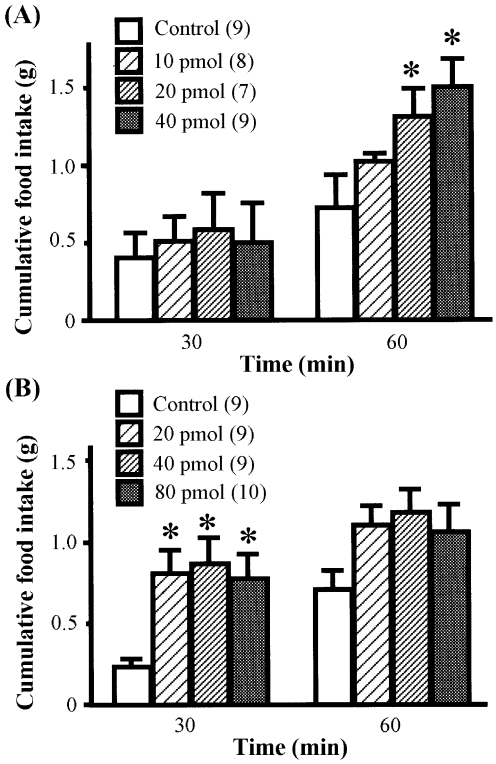

Fig. 2. Effects of intracerebroventricular injection of δ-opioid receptor agonists (A: DADLE; B: DPDPE) on food intake in chicks. Values are means ± S.E.M. of the number of chicks (shown in parentheses). * $P<.05$, compared with saline control.

[Fig. 3A: $F(4,94)=3.355$, $P<.05$; Fig. 3B: $F(4,60)=6.410$, $P<.05$].

4. Discussion

We found that intracerebroventricular injection of both μ agonists inhibited the feeding behavior of chicks (Fig. 1). Although there was no systematic attempt to quantify other behavioral measurement, μ agonists seemed to induce sleep-like behavior (sedation) in chicks during the first 30 min postinjection. The effect of DAMGO was especially strong, and the lower doses (10–20 pmol) also tended to decrease food intake by sedation, but not significantly (unpublished data). However, the possibility that the orexigenic effect of μ-opioid agonists, as it is in mammals (Gosnell and Levine, 1996), may be interrupted by sedation in chicks is remote because the intracerebroventricular administration of μ agonist (β-casomorphin 1-4, amide) had no effect on feeding behavior in broiler chickens (McCormack and Denbow, 1989). In addition, this discrepancy between chicks and chickens may be ascribed to the fact that different ages of chickens were used in these studies. It is known that chicks sleep much of the time, but with increasing age, the amount of time spent asleep declines (Roger, 1995). In fact, μ-opioid agonist produced a hypnotic effect in mammals (Reinoso-Barbero and De Andres, 1995). Thus, it is suggested that the regulation of sleep via μ-opioid receptors is important for neonatal chicks.

As for the effect of δ agonists, both were found to stimulate food consumption in neonatal chicks (Fig. 2). However, the response for DADLE (Fig. 2A) was different from that for DPDPE (Fig. 2B) because its effects were delayed relative to DPDPE. The possible reason for the delayed action of DADLE could be attributed to a sedative effect via μ receptor from the aforementioned result of μ agonist because DADLE also has an affinity to the μ receptor (Minami and Satoh, 1995). In early reports of opioid in chickens, intracerebroventricular administration of preferential δ agonists induced hyperphagia in broiler chickens (McCormack and Denbow, 1988, 1989; Savory et al., 1989). Thus, it is certain that δ agonists may act to induce hyperphagia in the central nervous system of chickens, regardless of age.

McCormack and Denbow (1989) reported that the intramuscular injection of both μ- and δ-opioid receptor agonists stimulates feeding behavior in chickens. Additionally, the blood–brain barrier may not yet be formed in the chicks used in this study. From these results, there is the possibility for peripheral agonist action on feeding behavior in neonatal chicks. However, we found that peripheral injection of DPDPE (400 pmol) did not enhance the feeding behavior of chicks (unpublished data). Therefore, the possibility that the intracerebroventricular injection of each agonist, at least

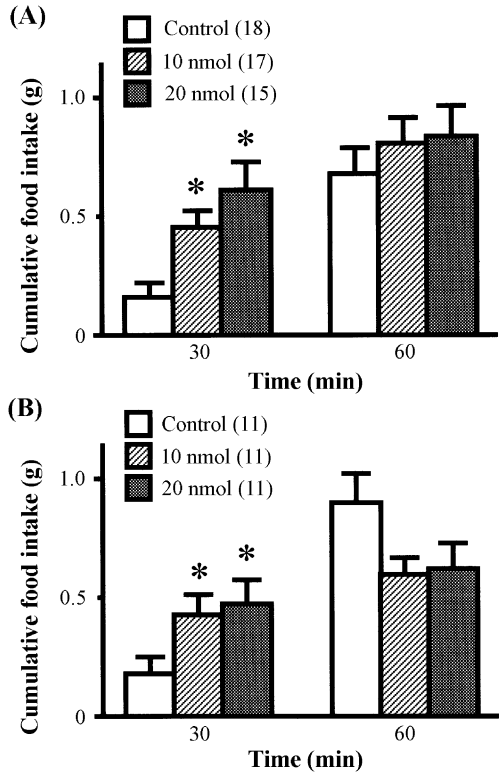

Fig. 3. Effects of intracerebroventricular injection of κ-opioid receptor agonists (A: U-50488H; B: U-62066) on food intake in chicks. Values are means ± S.E.M. of the number of chicks (shown in parentheses). * $P<.05$, compared with saline control.

DPDPE, acutely alters feeding behavior at sites outside the blood–brain barrier in chicks was low.

Central injections of specific κ agonists (U-50488H and U-62066) were found to elicit significant stimulation of feeding behavior in broiler chicks (Fig. 3), but a finding disagrees with that observed in 2-day-old Leghorn chicks using dynorphin (Steinman et al., 1987). The reason for this is not clear, but there are two possible explanations. First, the orexigenic effect of dynorphin via κ receptors might be interrupted by the sedative effect through μ receptors because dynorphin can slightly activate μ-opioid receptors, whereas κ-opioid agonists in the present study seldom act on them (Minami and Satoh, 1995). Second, strain may be important because layer-type chicks were used in the early study, whereas meat-type chicks were used in the present study. There are many reports that the effects of peptides or neurotransmitters on feeding behavior were influenced by genetic selection. For example, exendin (5–39) and GABA agonists stimulated the food intake of layer-type chicks but not meat-type chicks (Tachibana et al., 2001; Bungo et al., 2003).

In the present study, we used two agonists for each three-opioid receptor (μ-, δ- or κ-opioid receptor) and found the tendencies that each agonist indicated a slightly different duration in efficacy. Although there is no comparison study for each opioid receptor in chickens, it is likely that the differences with two agonists might contribute to each affinity for receptor, especially heteroreceptor (e.g., μ_1- and μ_2-receptor). It is known that each of the three receptors is subdivided into some heteroreceptors, and each heteroreceptor has different roles in mammals (e.g., Carvey, 1998). Clearly, further work for heteroreceptor is necessary in chickens. However, the results presented here suggest that δ- and κ-opioid receptor agonists induce hyperphagia, and the endogenous opioid peptides may play an important role in the feeding behavior of chicks. Additionally, further experiments will be required to determine the relationship between the orexigenic and sedative effects of opioids, especially through μ-opioid receptors.

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References

Akil J, Watson SJ, Young E, Lewis ME, Khachaturiuan H, Walker JM. Endogenous opioids: biology and function. Annu Rev Neurosci 1984;7:223–55.

Bodnar RJ. Opioid receptor subtype antagonists and ingestion. In: Cooper SJ, Clifton PG, editors. Drug receptor subtypes and ingestive behavior. London: Academic Press; 1996. p. 127–46.

Bungo T, Izumi T, Kawamura K, Takagi T, Ueda H, Furuse M. Intracerebroventricular injection of muscimol, baclofen or nipecotic acid stimulates food intake in layer-type, but not meat-type, chicks. Brain Res 2003;993:235–8.

Carvey PM. Opioid analgesics and pain. Drug action in the central nervous system. New York: Oxford Univ. Press; 1998. p. 75–104.

Csillag A, Bourne RC, Kalman M, Boxer MI, Stewart MG. Distribution of mu, delta, and kappa opioid receptor binding sites in the brain of the one-day-old domestic chick (*Gallus domesticus*). J Comp Neurol 1990;302:543–51.

Davis JL, Masuoka DT, Gerbrandt JF, Cherkin A. Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol Behav 1979;22:693–5.

D'amato FR. Neurobiological and behavioral aspects of recognition in female mice. Physiol Behav 1997;62:1311–7.

Gosnell BA, Levine AS. Stimulation of ingestive behaviour by preferential and selective opioid agonists. In: Cooper SJ, Clifton PG, editors. Drug receptor subtypes and ingestive behavior. London: Academic Press; 1996. p. 147–66.

Holtzman SG. Further characterization of the discriminative stimulus effects of spiradoline. Pharmacol Biochem Behav 2000;66:517–22.

Kanjhan R. Opioids and pain. Clin Exp Pharmacol Physiol 1995;22:397.

Koch G, Wiedemann K, Teschemacher H. Opioid activities of human beta-casomorphins. Naunyn-Schmiedeberg's Arch Pharmacol 1985;331:351–4.

Kotegawa T, Takahashi T, Tsutsui K, Ikeda T, Minakata H, Nomoto K. Isolation and characterization of opioid peptides in the avian brain. J Exp Zool 1995;273:87–95.

Martin R, McGregor GP, Halbinger G, Falke N, Voigt KH. Methionin^5-enkephalin and opiate binding sites in the neurohypophysis of the bird, Gallus domesticus. Regul Pept 1992;38:33–44.

McCormack JF, Denbow DM. Feeding, drinking and temperature response to intracerebroventricular β-endorphin in the domestic fowl. Peptides 1988;9:709–15.

McCormack JF, Denbow DM. Ingestive responses to mu and delta opioid receptor agonists in the domestic fowl. Br Poult Sci 1989;30:327–40.

Minami M, Satoh M. Molecular biology of the opioid receptors: structures, functions and distributions. Neurosci Res 1995;23:121–45.

National Research Council. Guide for the care and use of laboratory animals. Department of Health and Human Services. Washington, DC: NIH Publ. No. 85-23; 1985.

Reinoso-Barbero F, De Andres I. Effects of opioid microinjections in the nucleus of the solitary tract on the sleep–wakefulness cycle states in cats. Anesthesiology 1995;82:144–52.

Roger LJ. Early learning after hatching. The development of brain and behaviour in the chicken. Oxon: Cab International; 1995. p. 72–119.

Savory CJ, Gentle MJ, Yeomans MR. Opioid modulation of feeding and drinking in fowls. Br Poult Sci 1989;30:379–92.

Steinman JL, Fujikawa DG, Wasterlain CG, Cherkin A, Morley JE. The effects of adrenergic, opioid and pancreatic polypeptidergic compounds on feeding and other behaviors in neonatal leghorn chicks. Peptides 1987;8:585–92.

Tachibana T, Sugahara K, Ohgushi A, Ando R, Sashihara K, Yoshimatsu T, et al. Intracerebroventricular injection of exendin (5-39) increases food intake of layer-type chicks but not broiler chicks. Brain Res 2001;915:234–7.

Zhang C, Moss IR. Age-related mu-, delta- and kappa-opioid ligands in respiratory-related brain regions of piglets: effect of prenatal cocaine. Brain Res Dev Brain Res 1995;87:188–93.