Effects of N-terminal Fragments of β-Endorphin on Feeding in Chicks

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Abstract

It is known that N-terminal fragments of β-endorphin have biological activities, such as an antagonism effect of β-endorphin (1-31) on the secretion of hormones or thermoregulation in mammals. We studied the effects of the N-terminal fragments on feeding behavior in male broiler chicks. Intracerebroventricular administration of β-endorphin (1-27) (0.4 nmol) stimulated feeding behavior compared with saline control during the 60-min experimental period. β-endorphin (1-17) (2.0 nmol) also increased food intake at 30 min postinjection. Co-injection of either β-endorphin (1-27) or (1-17) was effective in reducing full-length β-endorphin-induced feeding in chicks. These data suggest that the N-terminal fragments of β-endorphin act as a partial agonist, and may regulate the activity of the central opioidergic system in chicks.

Keywords: β-endorphin; N-terminal fragment; Feeding behavior; Central nervous system; Chick

The role of the opioid peptides and their receptors in modulating feeding behavior has been a source of intense study in mammals [5]. Several reports implicate that, similar to mammals, opioid peptides play a facilitatory role in the ingestion of food in birds [10,16,18,21]. Recently, subsequent studies have investigated the opioid modulation of specific feeding-elucidated receptor mechanisms through the use of selective opioid receptor subtype agonists and antagonists in chicks [6-8]. In the context of these results, we also reported that one of the opioid peptide family, β-endorphin (β-EP), is involved in feeding behavior in chicks [27]. Therefore, it is considered that the opioidergic system plays an important role in feeding regulation in the central nervous system.

β-EP is derived from β-lipotropin, which in turn is derived from its precursor peptide, proopiomelanocortin [15,26]. It is known that produced β-EP is processed N-terminal fragment peptides by enzyme (e.g., γ-EP-generating enzyme for the Phe^{18}-Lys^{19} position [20] or prohormone convertase 2 for the Lys^{28}-Lys^{29} position [14]), and the processing patterns differ in various regions in the central nervous system of mammals [14,25,30,31]. Initially, these fragments were considered as an inactive form of β-endorphin [30]. Subsequently, several studies reported that the β-EP fragments also have low potency and attenuate β-EP induced action, such as analgesia, hypothermia and release of dopamine [13,21,26,27]. In contrast to these results in mammals, the functional significance of β-EP N-terminal fragments in controlling any behavior, especially feeding behavior, in avian species is unknown. The aim of this study is to elucidate whether central administration of β-EP N-terminal fragments modulates feeding behavior in the neonatal chick. A further experiment was undertaken to explore the interaction of β-EP with its N-terminal fragments.

Day-old male broiler chicks (Chunky) were purchased from a local hatchery (Fukuda chicken farm, Okayama, Japan). The birds were maintained in a room with

24-h lighting and at a temperature of 30°C. They were given free access to a commercial starter diet (Nichiwa Sangyo Co. Ltd., Kobe, Japan) and water during the pre-experimental period. 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 handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

β-EP-(1-31) (human) and β-EP-(1-27) were purchased from Sigma (St. Louis, MO, USA), and β-EP-(1-17) was obtained from Peptide Institute Inc (Osaka, Japan). The peptides were dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. Saline containing Evans Blue was used as a control. The birds were intracerebroventricularly (ICV) injected with the solutions (10 μl) using a microsyringe according to the methods used by Davis et al. [9]. Each chick was injected once only with either saline or peptide(s).

Birds (2- or 3-day-old) were given free access to food for 1 h immediately after each treatment. Food intake was determined by measuring the reduction of diet from a pre-weighed feeder. The weight of feeders was measured using an electric digital balance of precision ± 1 mg. In Experiment 1, birds were injected by ICV route with saline or β-EP-(1-27) (0.1, 0.2 or 0.4 nmol). In Experiment 2, saline or β-EP-(1-17) (0.5, 1.0 or 2.0 nmol) was injected once ICV into the lateral ventricle. In Experiment 3, chicks were injected with saline, β-EP-(1-31) (50 pmol), EP-31 co-injected with β-EP-(1-27) (0.1 nmol) or β-EP-(1-17) (1.0 nmol). The dose of β-EP-(1-31) was determined according to the previous report [25].

At the end of the experiments, chicks were sacrificed by decapitation, followed by brain sectioning to identify the location of the drug injection. Data were deleted for

individuals in which the presence of Evans Blue dye in the lateral ventricle was not verified. The number of birds used for data analysis is shown in each figure.

The data were analyzed using the commercially available package, StatView (Version 5, SAS Institute, Cary, USA, 1998). The Tukey-Kramer test was used to determine overall statistical significance due to treatment. Differences were considered to be significant when P was less than 0.05. Results are presented as means ± S.E.M.

The effect of ICV administration of β-EP-(1-27) on food consumption in broiler chicks fed ad libitum is shown in Fig. 1. The ICV injection of β-EP-(1-27) increased food consumption in a dose-dependent manner, and food intake increased significantly with 0.4 nmol of β-EP-(1-27) when compared with control over a period of 60 min (30 min: $F[3, 24]=3.436$, $P<0.05$; 60 min: $F[3, 24]=3.634$, $P<0.05$).

Figure 2 shows the effect of β-EP-(1-17) on feeding behavior in chicks after ICV injection. Food intake of the 2.0 nmol β-EP-(1-17) group was greater than that of the control group at 30 min postinjection ($F[3, 29]=4.775$, $P<0.01$), but the effect disappeared at 60 min ($F[3, 29]=2.778$, $P>0.05$).

The effect of ICV co-injection of each fragment on β-EP-(1-31)-induced hyperphagia in broiler chicks is shown in Fig. 3. Food intake of the β-EP-(1-31) group was greater than that of the control groups during the experimental period (30 min: $F[3, 31]=2.918$, $P<0.05$; 60 min: $F[3, 31]=5.159$, $P<0.01$). Although the differences were not significant when compared with the use of EP-31 alone, co-injection of β-EP-(1-27) (0.1 nmol) showed a tendency to attenuate the β-EP-(1-31)-induced eating response over a period of 60 min. Co-injection of β-EP-(1-17) (1.0 nmol) also tended to block the orexigenic effect of β-EP-(1-31) at 30 min postinjection. In the 0–60 min feeding interval, β-EP-(1-17) significantly attenuated the eating response induced by β-EP-(1-31).

The results of the present study show that both fragments act as an orexigenic agent in chicks (Figs. 1 and 2). The results of this and previous studies [25] indicate that the rank order of potency on feeding behavior is β-EP-(1-31) > β-EP-(1-27) > β-EP-(1-17). This suggests that each fragment might bind the opioid receptor, but the efficacy was reduced with the decreasing number of amino acids of the peptide. Similar to these results, Furuse et al. [10] showed that N-terminal fragments of glucagon-like peptide-1 (7-36) had a lower potency than the original peptides.

It is known that N-terminal amino acids play an important role in affinity for the receptor of peptide hormones, including opioids [1,12,23,28]. Alt *et al.* [3] revealed that the affinity of β-EP-(1-27) for the opioid receptor is similar to that of full length β-EP. Previously, we found that β-EP-(1-31)-induced hyperphagia was attenuated by μ-, but not δ-opioid receptor antagonist [29]. Thus, the orexigenic effect of ~~these fragments~~ β-EP-(1-27), having the same amino acid sequences in the N-terminal, could mediate via the μ-opioid receptor in the central nervous system of chicks. On the other hand, not only the affinity of β-EP-(1-17) for the opioid receptors is reduced but also selectivity to the μ-opioid receptor [22]. β-EP-(1-17)-induced feeding is also mediated by the δ-opioid receptor is a factor that is not refuted.

We found that an ineffective dose of each fragment on feeding behavior attenuated the orexigenic effect of full-length β-EP (Fig. 3). The attenuation might be caused by competition between β-EP-(1-31) and each N-terminal fragment. Even if selectivity of β-EP-(1-17) to the δ-opioid receptor increased, the possibility that the stimulation via the δ-opioid receptor as the orexigenic signal attenuates β-EP-(1-31)-induced feeding was low. Thus, the N-terminal fragments might act as a partial agonist for the μ-opioid receptor. In mammals, it is reported that β-EP-(1-27) attenuated β-EP-(1-31)-induced hypothermia [27], analgesia [13,21] and release of dopamine [26], and β-EP-(1-27) blocks the effects of μ- and δ-opioid agonists [4].

Interestingly, the potency of β-EP-(1-27) is 4–5 times greater than that of the opiate antagonist naloxone [18]. In any case, the results described here ~~is~~ are the first report about attenuation of the N-terminal fragments on β-EP-induced feeding in chicks.

It is reported that β-EP-(1-31) processing patterns are different in various brain regions [25,31], and changed by stress in mammals [2,18]. Although we did not investigate where β-EP fragments are distributed in the brain of chicks, the results in mammals indicate that naturally occurring β-EP-fragments have biological significance in the central nervous system [29,30].

In conclusion, the N-terminal fragments of β-EP, as well as full length β-EP, may have an important role in the regulation of feeding behavior and the biological activity of β-EP acting as a partial agonist in the central nervous system of chicks. Further experiments will be required to determine the biological role of the various truncated forms of β-EP, and the interaction between the opioid active and partial agonist forms of β-EP in the chick.

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Legends

Fig. 1. Cumulative food intake of chicks injected ICV with saline or one of three doses (0.1, 0.2 or 0.4 nmol) of β-endorphin (1-27). Values are means ± SEM of the number of chicks in parentheses. *P<0.05, compared with saline control. Food intake (g/30 min)=0.415+1.656X (R^2=0.25, P<0.01), food intake (g/60 min)=0.816+1.393X (R^2=0.24, P<0.01).

Fig. 2. Cumulative food intake of chicks injected ICV with saline or one of three doses (0.5, 1.0 or 2.0 nmol) of β-endorphin (1-17). Values are means ± SEM of the number of chicks in parentheses. *P<0.05, compared with saline control. Food intake (g/30 min)=0.342+0.184X (R^2=0.27, P<0.01).

Fig. 3. Effect of β-endorphin (1-27) (0.1 nmol) or β-endorphin (1-17) (1.0 nmol) on β-endorphin (1-31) (50 pmol) induced feeding in chicks. EP 31: β-endorphin (1-31), EP 27: β-endorphin (1-27), EP 17: β-endorphin (1-17). Values are means ± SEM of the number of chicks in parentheses. Means with different letters are significantly different at p<0.05.

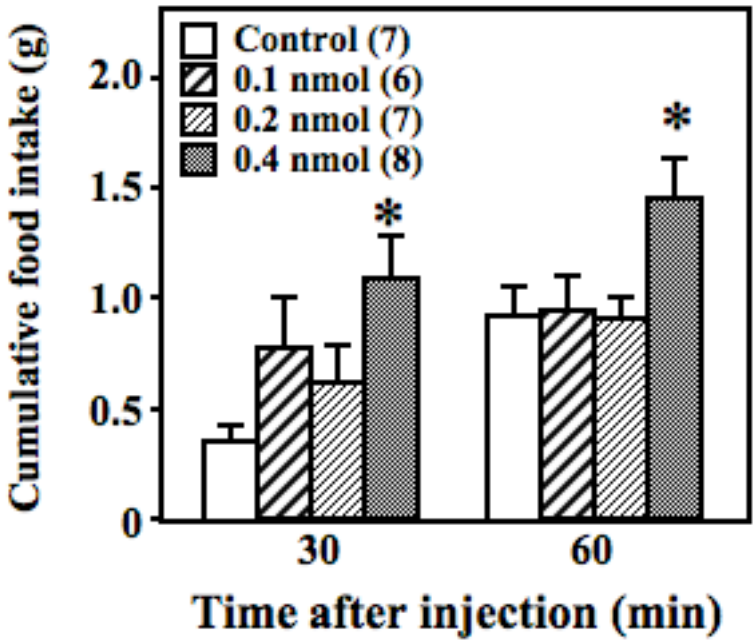
Control (7)
0.1 nmol (6)
0.2 nmol (7)
0.4 nmol (8)
Cumulative food intake (g)
2.0
1.5
1.0
0.5
0
*
*
30
60
Time after injection (min)

Fig. 1

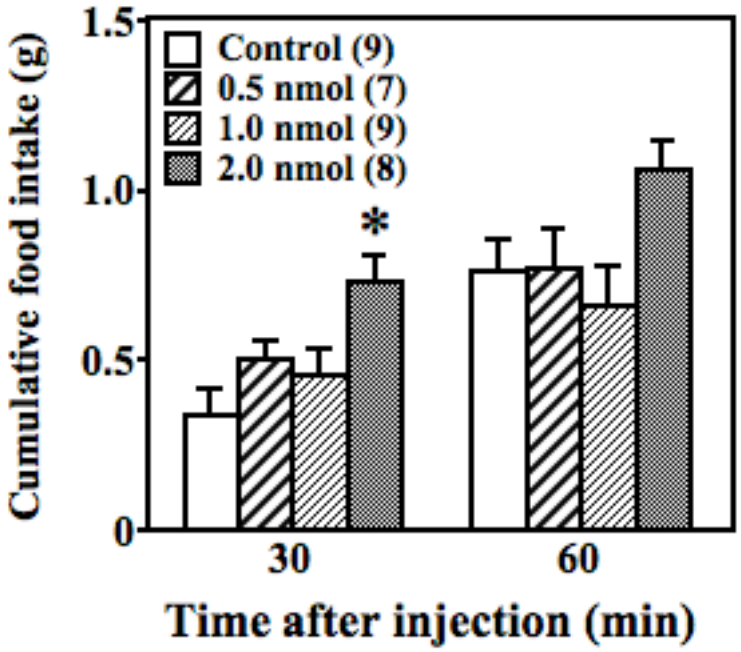
Cumulative food intake (g)
1.5
1.0
0.5
0
Control (9)
0.5 nmol (7)
1.0 nmol (9)
2.0 nmol (8)
*
30
60
Time after injection (min)

Fig. 2

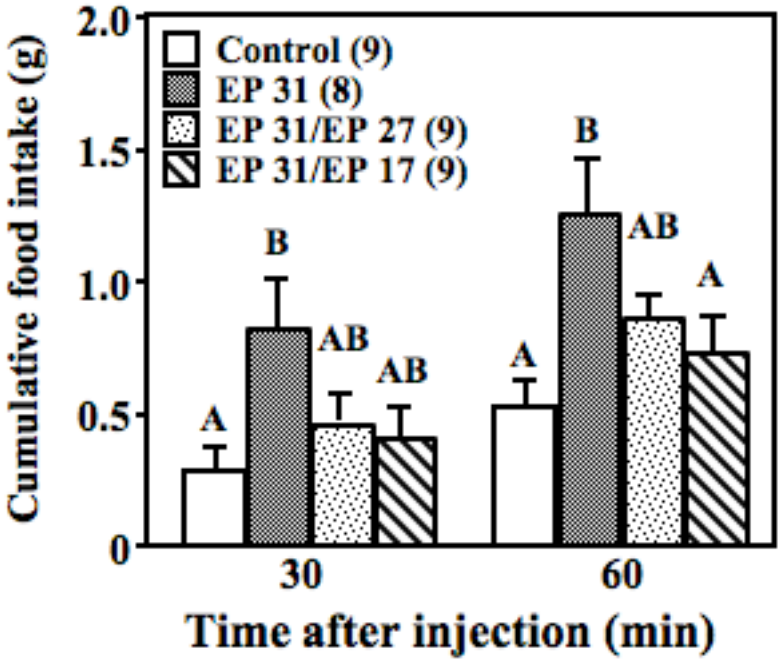
Cumulative food intake (g)
2.0
1.5
1.0
0.5
0
Control (9)
EP 31 (8)
EP 31/EP 27 (9)
EP 31/EP 17 (9)
A
B
AB
AB
A
B
AB
A
30
60
Time after injection (min)

Fig. 3