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Central Injections of β-Endorphin Fragment Modulate the Anorexia by Insulin in Neonatal Chicks

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Abstract: Recently, researchers found that β-endorphin regulates the activity of the central melanocortin system and its activation provides an inhibitory feedback mechanism in the brain of neonatal chicks. It is known that fragments of β-endorphin have biological activities. Thus, the present study was done to elucidate whether N- or C-terminal of β-endorphin fragment affects the insulin-induced anorexia in chicks. Researchers found that intracerebroventricular injection of insulin with N-terminal fragment, β-endorphin-(1-27), accelerated the insulin-induced hypophagia in neonatal chicks during the 60 min period postinjection. Conversely, the anorexic effect of insulin was attenuated by C-terminal fragment, β-endorphin-(30-31). These data suggest that both fragments involve in the regulation of feeding behavior and they may modulate the activity of the central melanocortin system in the brain of neonatal chicks.

Key words: Feed intake, β-endorphin, fragment, insulin, central nervous system, chick

INTRODUCTION

Much evidence has been collected suggesting an importance of the central melanocortin system in the regulation of feeding behavior (Schwartz *et al*., 2000). It is known that this system is the downstream mediator of insulin that acts in the brain to reduce feed intake and body weight (Woods *et al*., 1998; Benoit *et al*., 2002). Recently, researchers found that insulin functions as an appetite-suppressive peptide in the central nervous system of chicks and that this anorexic effect of insulin was due to α-Melanocyte Stimulating Hormone (α-MSH), a post-translational processing of Pro-Opiomelanocortin (POMC) within this system (Shiraishi *et al*., 2008a). POMC is also precursor of β-endorphin (Takeuchi *et al*., 1999; Gerets *et al*., 2000; Mains *et al*., 1977).

β-Endorphin acts in an autoreceptor manner to the μ-opioid receptor on POMC neurons, diminishing POMC neuronal activity in response to elevated POMC-derived melanocortin peptides that inhibited feed intake in mammals (Cowley *et al*., 2001; Wardlaw *et al*., 1996). Similar to mammals, it is reported that β-endorphin regulates the activity of the central melanocortin system and its activation provides an inhibitory feed back mechanism in the brain of neonatal chicks (Shiraishi *et al*., 2008b).

It is known that produced β-endorphin is processed fragment peptides by enzyme and the processing patterns differ in various regions in the central nervous system of mammals (Loh, 1992; Smyth, 1983). These fragments were considered as an modulator for the endorphinergic system (Zakarian and Smyth, 1979). In contrast to these results in mammals, the functional significance of β-endorphin fragments in controlling the melanocortin system in avian species is unknown.

The aim of this study is to elucidate whether central administration of β-endorphin N- or C-terminal fragment modulates the insulin-induced anorexia in the neonatal chicks.

MATERIALS AND METHODS

Day old male layer-type chicks (single comb white leghorn) were obtained from a local hatchery (Akita Co. Ltd, Hiroshima, Japan) 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

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Committee of Hiroshima University. Porcine insulin was purchased from MP Biomedicals, Inc. (Auroa, OH, USA) and β-endorphin-(1-27) and β-endorphin-(30-31) was obtained SIGMA (St. Louis, MO, USA). 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.* (1979). Each chick was injected once only with either saline or peptide (s).

Birds (2 or 3 days 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, insulin (20 ng) or insulin co-injected with β-endorphin-(1-27) (200 pmol). In Experiment 2, saline, insulin (20 ng) or insulin co-injected with β-endorphin-(30-31) (100 nmol) was injected once ICV into the lateral ventricle.

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 legend.

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

RESULTS AND DISCUSSION

Figure. 1 shows the effect of β-endorphin-(1-27), the N-terminal fragment on feeding behavior in chicks after co-injection of insulin. Feed intake of the 200 pmol β-endorphin-(1-27) co-injection group was lower than that of the control group at 60 min postinjection ($p<0.01$). It is reported that β-endorphin-(1-27) antagonized β-endorphin-(1-31)-induced hypothermia (Suh *et al.*, 1987), analgesia (Nicola and Li, 1985) and release of dopamine (Spanagel *et al.*, 1991) and β-endorphin-(1-27) antagonizes the effects of μ-opioid agonists (Bals-Kubik *et al.*, 1988). Including β-endorphin-(1-27), the N-terminal fragments of β-endorphin have low potency and attenuate β-endorphin-(1-31)-induced action via specific opioid receptor in the central nervous system of chicks (Yanagita *et al.*, 2008). Because β-endorphin-(1-31) attenuates the insulin-induced anorexia (Shiraishi *et al.*, 2008b), the present result implied that the N-terminal fragment might antagonize the endogenous β-endorphin in the central nervous system of chicks. Consequently, it seemed that the hypophagic effect of insulin was accelerated by co-injection of β-endorphin-(1-27).

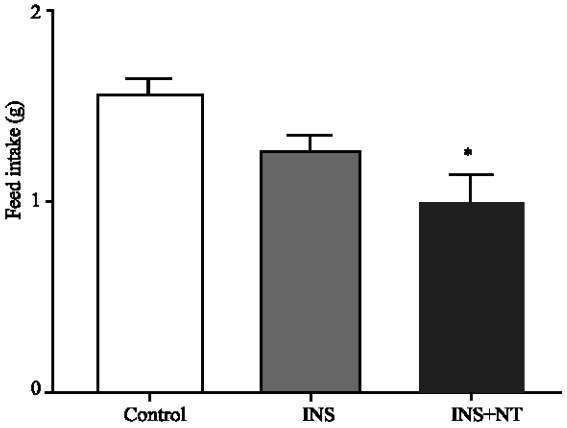

Fig. 1: Food intake during the 60 min period postinjection of fasted chicks intracerebroventricularly injected with saline, insulin (INS; 20 ng) or INS co-injected with β-endorphin-(1-27), the N-terminal fragment (NT; 200 pmol). The number of chicks in each group was as follows: control, 9; INS alone, 9; INS+NT, 8. Data are expressed as means±SEM. *$p<0.05$ compared with saline control

The effect of ICV administration of insulin with β-endorphin-(30-31), the C-terminal fragments on feed consumption in fasted chicks is shown in Fig. 2. Feed intake decreased significantly with 20 ng of insulin ($p<0.05$) and co-injection of the C-terminal fragment attenuated the insulin-induced hypophagia during the 60 min period postinjection.

Recently, researchers found that insulin functions as an appetite-suppressive peptide in the central nervous system of chicks and that this anorexic effect of insulin was due to α-Melanocyte Stimulating Hormone (α-MSH), a post-translational processing of Pro-Opiomelanocortin (POMC) via melanocortin 4 receptor (Shiraishi *et al.*, 2008a).

It has been proposed that α-MSH acts as multifunctional hormone, such as thermoregulation, inflammation, cardiovascular function and reproduction in mammals (Seeley *et al.*, 2004). Resch and Millington (1993) reported that β-endorphin-(30-31) inhibits α-MSH-induced thermogenesis when injected directly into hypothalamus, implying that β-endorphin-(30-31) diminishes POMC neuronal activity in response to elevated α-MSH.

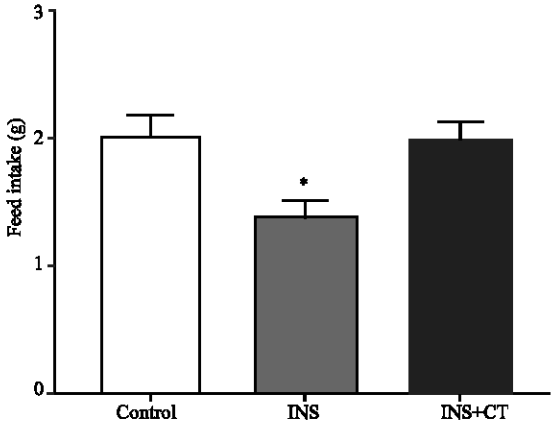

Fig. 2: Food intake during the 60 min period postinjection of fasted chicks intracerebroventricularly injected with saline, insulin (INS; 20 ng) or INS co-injected with β-endorphin-(30-31), the C-terminal fragment (CT; 100 nmol).. The number of chicks in each group was as follows: control, 6; INS alone, 7; INS+CT, 10. Data are expressed as means±SEM. *$p<0.05$ compared with saline control

Further research on the interaction of central melanocortin system and fragments of β-endorphin on feed regulation is necessary in neonatal chicks.

CONCLUSION

These data indicate that both N- and C-terminal fragments of β-endorphin involve in the regulation of feeding behavior and they may modulate the activity of the central melanocortin system, especially POMC neurons in neonatal chicks.

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