Opioid-induced hypophagia is mediated by 5-HT$_2$c receptors in neonatal layer-type chicken

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ABSTRACT: Opioidergic and serotonergic (5-HTergic) systems have crucial role in central regulation of food intake. This study was designed to investigate the role of the opioidergic system and the interaction with the 5-HTergic system in opioid-induced feeding behaviour in 3-h food-deprived (FD$_3$) neonatal layer-type chicks. In total 432 chickens were allocated into 9 experiments, each per 4 treatment groups. In Experiment 1, birds were intracerebroventricularly (ICV) injected with D-Ala2–NMe-Phe4-Glyol5-enkephalin (DAMGO), μ-opioid receptor agonist (125, 250, and 500 pmol). In Experiment 2, chickens were ICV treated with D-Pen^2, D-Pen^{5}enkephalin (D-Pen^{2,5}enkephalin, DPDPE), δ-opioid receptor agonist (20, 40, and 80 pmol). In Experiment 3, the effect of ICV injection of U-50488H, κ-opioid receptor agonist (10, 20, and 30 nmol) was investigated in chicks. In Experiment 4, chickens were injected with para-chlorophenylalanine (PCPA), cerebral serotonin depletive (1.5 μg) + DAMGO (125 pmol). In Experiment 5, birds were treated with PCPA (1.5 μg) + DPDPE (40 pmol). In Experiment 6, birds received 1.5 μg PCPA + U-50488H (30 nmol). In Experiments 7–9, birds were injected like in Experiments 4–6, but with SB242084, 5-HT$_2$c receptor antagonist (1.5 μg) instead of the PCPA injection. Cumulative food intake was recorded until 3 h post injection. According to the results, the ICV injection of DAMGO significantly decreased whereas that of DPDPE + U-50488H increased food intake ($P \leq 0.05$). Co-administration of PCPA + DAMGO significantly decreased hypophagia induced by DAMGO ($P \leq 0.05$). PCPA had no effect on DPDPE + U-50488H-induced hyperphagia ($P \geq 0.05$). SB242084 significantly attenuated the hypophagic effect of DAMGO ($P \leq 0.05$), while SB242084 had no modulatory effect on the food intake induced by DPDPE + U-50488H ($P \geq 0.05$). These results suggest that there is an interaction between the opioidergic and 5-HTergic systems mediating the hypophagic effect of μ-opioid receptors via the 5-HT$_2$c receptor in neonatal layer-type chicks.

Keywords: opioidergic; 5-HTergic system; food intake; layer-type chicks

INTRODUCTION

The appetite in birds is controlled by diverse neurotransmitters via complex neurological pathways (Zendehdel et al. 2014). Serotonin (5-hydroxytryptamine, 5-HT) is a classic monoamine neurotransmitter which is involved in numerous physiological functions, e.g. sleep-wake, circadian rhythm, motor control, nociception, immune system, behaviour and mood, anxiety, aggressiveness, and depression (Nonogaki et al. 2008, 2009;

Zendehdel et al. 2013a). The 5-HTergic neurons are localized in clusters with the raphe nuclei, central gray and reticular formation in the central nervous system (CNS). Seven main families of 5-HT receptors (5-HT$_1$–5-HT$_7$) belonging to G protein-coupled receptors (GPCRs) have been identified (Idova et al. 2012). The central 5-HTergic system has a potential effect on food intake. Reportedly the intracerebroventricular (ICV) injection of 5-HT has a hypophagic effect on food intake in chicken (Zendehdel et al. 2012a, b, 2013a, b, 2014).

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Opioids are known as inhibitory neurotransmitters. Three main opioid receptors were classified: mu (μ), delta (δ), and kappa (κ) which are homologous to GPCRs (Le Merrer et al. 2009). Opioid receptors coincide with many areas of the CNS that regulate ingestion including the hypothalamus, nucleus accumbens (NAcc), amygdala, ventral tegmental area (VTA), and nucleus of the tractus solitaries (NTS) (Barnes et al. 2006). The central opioidergic system is associated with pain perception, respiration, and immune system (Solati 2011).

Recently, a considerable attention has been given to the role of central opiates in appetite (Kozlov et al. 2013). Reports on the involvement of endogenous opiates in animal food intake have yielded controversial results. For instance, the ICV injection of μ- and δ- (but not κ-) opioid receptors agonists exerts orexigenic effects in mammals (Taha 2010; Kaneko et al. 2012). The ICV injection of μ-opioid receptors agonist decreases while that of δ- and κ-opioid receptors agonists increases food intake in neonatal meat-type chicks (Bungo et al. 2005). Inter-species differences existing between animals suggest that food intake is increased via μ-opioid receptor in broilers (Dodo et al. 2005; Shiraishi et al. 2008).

The neuroendocrine control of food intake and energy balance is a complex process controlled by many overlapping integrated pathways. There is a possible interaction between endogenous opioids and other neurotransmitters (Echo 2004; Villa et al. 2008; Guy et al. 2011). The neurological relationship between the hypothalamic 5-HTergic and opioidergic systems under opioids administration stimulates the 5-HT synthesis and metabolism (Robert et al. 1991). For instance, cocaine inhibits 5-HT and norepinephrine reuptake into presynaptic nerve terminals (Di Benedetto et al. 2004). Co-injection of 5-HT receptor agonists and μ-opioid agonist has antinociceptive effects (Li et al. 2011).

Arcuate nucleus (ARC) contains numerous neuropeptides and a variety of ligands for the receptors that can influence energy expenditure (Shiraishi et al. 2008). It is reported that endogenous cocaine/amphetamine regulated transcript (CART) peptide regulates synthesis of μ-opioid and 5-HT$_2$ receptors (Rothman et al. 2003). Endogenous opioids neurons were identified in raphe nucleus, where 5-HT are the main neurotransmitter (Kirby et al. 2011). There is no evidence on the effect of the relationship of the 5-HTergic system and different opiate receptors on appetite. In addition, the effects of ICV injection on 5-HT and/or opioidergic systems in avian species have been scarcely studied. Based on the literature, the objective of the current study was to investigate the possible effect of the interaction between the central opioidergic and 5-HTergic systems on the feeding behaviour in neonatal layer-type chicks.

MATERIAL AND METHODS

Animals. One-day-old Bovans layer-type chickens (Seamorgh Co., Iran) were kept in a flock for 2 days, then randomly transferred into individual cages, and kept at 30 ± 1°C and 50 ± 2% humidity (Olanrewaju et al. 2006). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (USA) and the current laws of the Iranian government for animal care. Commercial feed (Animal Science Research Institute Co., Iran) fed to chicks during the study contained 21% crude protein and 2850 kcal/kg metabolizable energy. All chickens received *ad libitum* feed and fresh water during the study. Prior to the injections, animals were food deprived for 3 h (FD$_3$), but had free access to water. ICV injections were applied to all birds on day 5 of age.

Experimental drugs. Para-chlorophenylalanine (PCPA) (cerebral 5-HT depletive), SB242084 (5-HT$_2$c receptor antagonist), DAMGO (μ-opioid receptor agonist), DPDPE (δ-opioid receptor agonist), and U-50488H (κ-opioid receptor agonist) were purchased from Sigma-Aldrich (St. Louis, USA). The drugs were dissolved in 0.1% Evans blue solution, which was prepared in either 0.85% saline or first dissolved in absolute dimethyl sulfoxide (DMSO) and then diluted with saline containing Evans blue, which was also used as the control solution.

ICV injection procedure. Before the initiation of each experiment, birds were weighed and based on their body weight allocated into experimental groups. So the mean body weight between treatment groups was as uniform as possible. To investigate the effect of the interaction of 5-HTergic and opioidergic systems on food intake in layer-type chicken, 9 experiments were designed, each including 4 treatment groups with 12 replicates in each (*n* = 48 chickens in each experiment;

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totally 432 birds in this study). ICV injection was applied using a microsyringe (Hamilton, Biel/Bienne, Switzerland) without anesthesia according to the technique described by Davis et al. (1979) and Furuse et al. (1997). Each bird's head was held with an acrylic device while the bill holder was at a 45° position and calvarium parallel to the surface of the table (Van Tienhoven and Juhasz 1962). A hole was drilled in the acrylic plate put over the right lateral ventricle of the skull. A microsyringe was inserted into the right ventricle via the hole and tip of the needle penetrated 4 mm beneath the skin of the skull. There is no injection-induced physiological stress using this method in neonatal chicks (Saito et al. 2005). To identify the precision of the injection into the ventricle, chicks were euthanized using overdose ether at the end of each experiment and the presence of Evans blue in slices of the frozen brain tissue was an indicator of the injection accuracy. Twelve chicks from each group were injected, however only data from those animals in the lateral ventricle of which the dye was detected (9–12 birds in each group) were used for analysis. Experiments were performed from 9:00 till 13:00 h.

Feeding experiments. In this study, 9 experiments were designed, each with 4 treatment groups: A, B, C, and D (n = 12 animals per group).

In Experiment 1, chicks received ICV injection as follows: (A) control solution (10 µl), (B) DAMGO, µ-opioid receptor agonist (125 pmol/10 µl), (C) DAMGO (250 pmol/10 µl), (D) DAMGO (500 pmol/10 µl).

In Experiment 2, birds were injected with (A) control solution (10 µl), (B) DPDPE, δ-opioid receptor agonist (20 pmol/10 µl), (C) DPDPE (40 pmol/10 µl), (D) DPDPE (80 pmol/10 µl).

In Experiment 3, animals received (A) control solution (10 µl), (B) U-50488H, κ-opioid receptor agonist (10 nmol/10 µl), (C) U-50488H (20 nmol/10 µl), (D) U-50488H (30 nmol/10 µl).

In Experiment 4, ICV injections were as follows: (A) control solution (10 µl), (B) PCPA, cerebral 5-HT depletive (1.5 µg/10 µl), (C) DAMGO, µ-opioid receptor agonist (125 pmol/10 µl), (D) 1.5 µg PCPA/5 µl + 125 pmol DAMGO/5 µl.

In Experiment 5, the birds received (A) control solution (10 µl), (B) PCPA (1.5 µg/10 µl), (C) DPDPE, δ-opioid receptor agonist (40 pmol/10 µl), (D) 1.5 PCPA/5 µl + 40 pmol DPDPE/5 µl.

In Experiment 6, ICV injections included: (A) control solution (10 µl), (B) PCPA (1.5 µg/10 µl), (C)

U-50488H, κ-opioid receptor agonist (30 nmol/10 µl), (D) 1.5 µg PCPA/5 µl + 30 nmol U-50488H/5 µl.

Birds in Experiments 7–9 followed the procedure similar to Experiments 4–6 except that chickens received an ICV injection of 1.5 µg SB242084 (5-HT$_2$c receptor antagonist) in place of PCPA. The applied injections were of 10 µl volume. In all experiments, control group received ICV injection of 10 µl saline (Evans blue). Each chick was injected only once. Right away after injection, birds were put back to their individual cages, given *ad libitum* feed and water. Then, cumulative food intake was recorded at 30, 60, 120, and 180 min post injection. Food consumption was recorded as percent of body weight (% BW) (to adjust differences between body weights) calculated on the amount of food intake from pre-weighed feeder. The doses of drugs applied in this experiment were chosen according to previous and pilot studies (Bungo et al. 2004, 2005; Khan et al. 2009; Zendehdel et al. 2013, 2014).

Statistical analysis. Cumulative food intake was analyzed by One-Way Analysis of Variance (ANOVA) in Experiments 1–3 and by Two-Way ANOVA for repeated measurement in Experiments 4–9 using the SPSS software (Version 16.0, 2006) for MS Windows and results are presented as means ± SEM. In the treatment with main effect indicated by ANOVA, means were compared by Duncan's test. $P \leq 0.05$ was considered as significant differences between treatments.

RESULTS

The effect of µ-, δ-, and κ-opioid receptors agonists on food intake in neonatal layer-type chickens is presented in Figures 1–3. As seen in Figure 1, the ICV injection of DAMGO (125, 250, and 500 pmol) significantly decreased cumulative food intake compared to control group at 30, 60, 120, and 180 min post-injection ($P \leq 0.05$). According to the results, food intake decreases via µ-opioid receptors in FD$_3$ neonatal layer-type chickens.

The effect of ICV injection of DPDPE (δ-opioid receptor agonist) on cumulative food intake in FD$_3$ neonatal layer-type chicks is shown in Figure 2. Based on the data, the ICV injection of 20 and 80 pmol DPDPE had no significant effect on food intake in comparison with control group ($P \geq 0.05$). Interestingly, 40 pmol of DPDPE significantly increased cumulative food intake (% BW) compared to control group ($P \leq 0.05$).

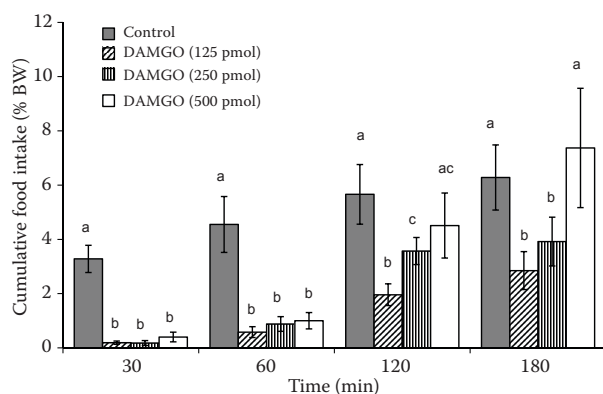*Czech J. Anim. Sci.*, 60, 2015 (9): 400–410

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Figure 1. Effect of ICV injection of different levels of μ-opioid receptor agonist (DAMGO) on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a–c) in a column ($P \leq 0.05$)
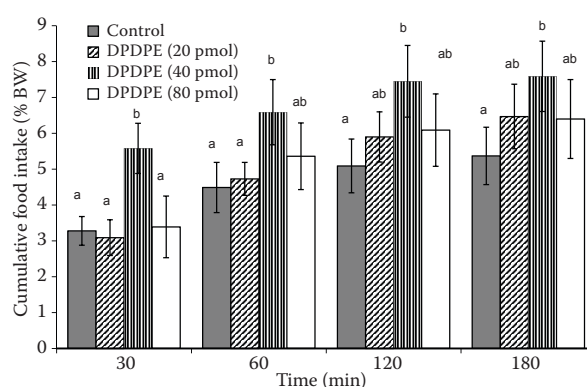

Figure 2. Effect of ICV injection of different levels of δ-opioid receptor agonist (DPDPE) on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)

In Experiment 3, we determined the effect of κ-opioid receptor agonist on cumulative food intake in FD_3 neonatal layer-type chicks. As seen in Figure 3, the ICV injection of U-50488H (κ-opioid receptor agonist) at doses of 10 and 20 nmol had no significant effect on food intake compared to control group ($P \geq 0.05$). Likewise, the ICV injection of 30 nmol U-50488H significantly increased food intake compared to control group ($P \leq 0.05$).

The effect of serotonergic system as well as its interaction with opioidergic system (μ-, δ-, and κ-receptors) on cumulative food intake in FD_3 neonatal layer-type chickens is presented in Figures 4–9. According to the results, the ICV injection of 1.5 μg PCPA (cerebral 5-HT depletive) had no significant effect on food intake (% BW) compared to control group at each time point in FD_3 neonatal layer-type chickens ($P \geq 0.05$). Hence, the ICV administration of DAMGO (μ-opioid receptor agonist) significantly diminished cumulative food intake in comparison to control group ($P \leq 0.05$). Also, co-administration of PCPA and DAMGO significantly blocked hypophagia induced by DAMGO ($P \geq 0.05$) (Figure 4). No sedation effect was observed in birds because in this study we used sub-effective doses of the drugs blocking the receptor but unaffecting food intake to investigate the possible interaction of μ-opioid receptor and 5-HTergic system on food intake. It seems that the effect of central μ-opioid receptor on food intake is mediated via 5-HTergic system in neonatal layer-type chickens.

The effect of the ICV injection of PCPA and δ-opioid receptor agonist on cumulative food intake in FD_3 neonatal layer-type chickens is presented in Figure 5. The ICV injection of PCPA had no significant effect on food intake in neonatal layer-type chickens ($P \geq 0.05$). The ICV injection of 40 pmol DPDPE (δ-opioid receptor agonist) significantly increased food intake in FD_3 neonatal layer-type chickens compared to control group ($P \leq 0.05$). Co-administration of PCPA with DPDPE had no significant effect on δ-opioid receptor induced hyperphagia ($P \geq 0.05$) (Figure 5). Presumably, 5-HTergic system played no role in δ-opioid re-
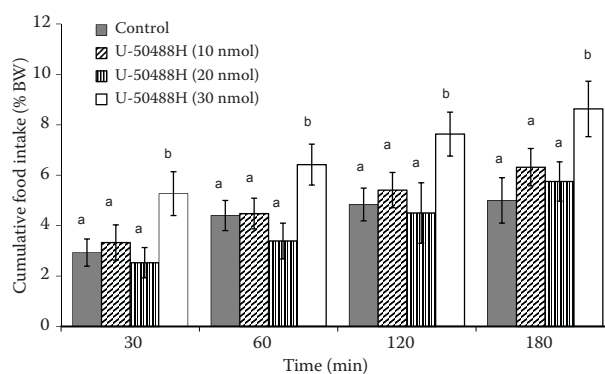

Figure 3. Effect of ICV injection of different levels of κ-opioid receptor agonist (U-50488H) on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)
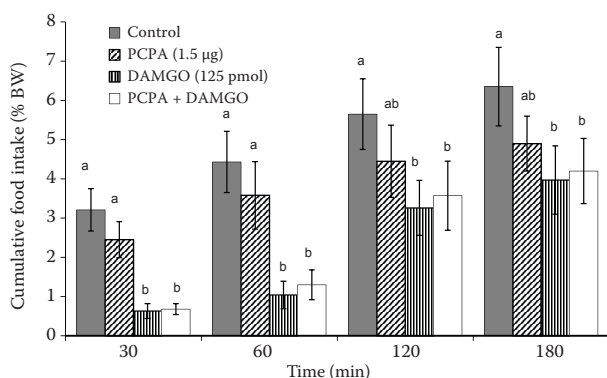
Figure 4. Effect of ICV injection of cerebral 5-HT depletive (PCPA, 1.5 μg) + μ-opioid receptor agonist (DAMGO, 125 pmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a.b) in a column ($P \leq 0.05$)

ceptor induced hyperphagia in neonatal layer-type chickens.

In Experiment 6, the interaction between 5-HTergic system and κ-opioid receptor on feeding behaviour in FD$_3$ neonatal layer-type chickens was studied. Figure 6 shows that the injection of PCPA had no effect on appetite ($P \geq 0.05$) whereas administration of 30 nmol U-50488H (κ-opioid receptor agonist) significantly amplified food intake compared to control group in FD$_3$ neonatal layer-type chickens ($P \leq 0.05$). Furthermore, κ-opioid

Figure 5. Effect of ICV injection of cerebral 5-HT depletive (PCPA, 1.5 μg) + δ-opioid receptor agonist (DPDPE, 40 pmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)

receptor-induced hyperphagia was not affected by PCPA until 3 h post injection ($P \geq 0.05$) (Figure 6). Perhaps, there was no interaction between central κ-opioid receptor and 5-HTergic system in neonatal layer-type chickens.

In Experiments 7–9, we investigated the interaction of 5-HT$_2$c receptors and opioidergic system on feeding behaviour. Based on the data (Figure 7), the ICV injection of 5-HT$_2$c receptor antagonist (SB242084; 1.5 μg) had no significant effect on cumulative food intake compared to control
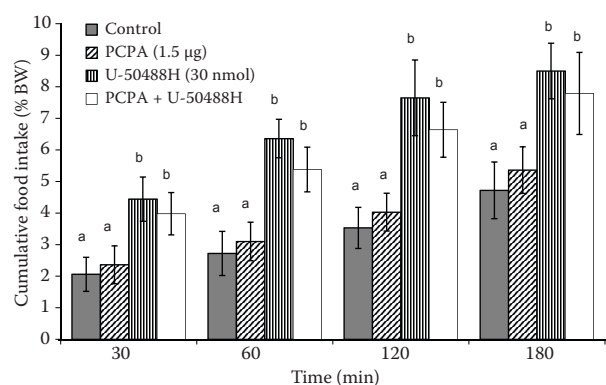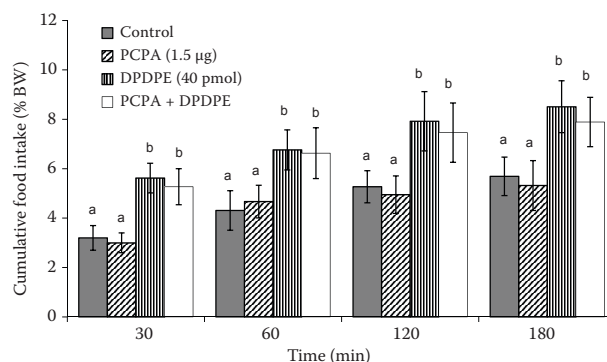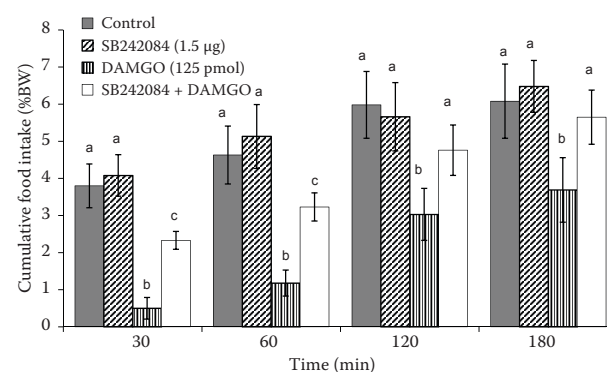

Figure 6. Effect of ICV injection of cerebral 5-HT depletive (PCPA, 1.5 μg) + κ-opioid receptor agonist (U-50488H, 30 nmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)

Figure 7. Effect of ICV injection of 5-HT$_2$c receptor antagonist (SB242084, 1.5 μg) + μ-opioid receptor agonist (DAMGO, 125 pmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a–c) in a column ($P \leq 0.05$)
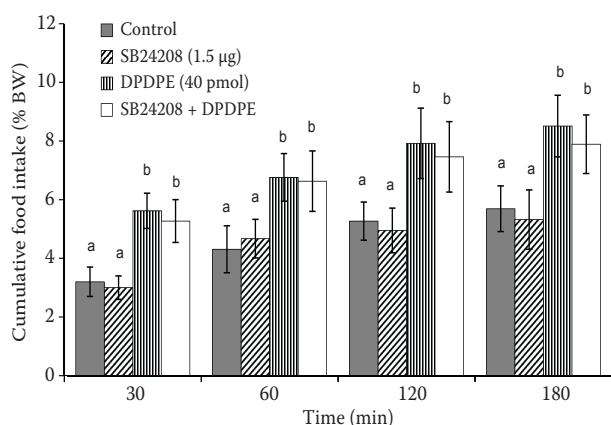
Figure 8. Effect of ICV injection of 5-HT$_2$c receptor antagonist (SB242084, 1.5 µg) + δ-opioid receptor agonist (DPDPE, 40 pmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)

Figure 9. Effect of ICV injection of 5-HT$_2$c receptor antagonist (SB242084, 1.5 µg) + κ-opioid receptor agonist (U-50488H, 30 nmol), and their combination on cumulative food intake (% BW) in neonatal layer type chickens

results are presented as mean ± SEM, there are significant differences between groups with different superscripts (a, b) in a column ($P \leq 0.05$)

group ($P \geq 0.05$). In contrast, administration of µ-opioid receptor agonist (DAMGO, 125 pmol) significantly reduced food intake in comparison to control group in FD$_3$ neonatal layer-type chickens ($P \leq 0.05$). Interestingly, co-injection of 5-HT$_2$c receptor antagonist plus µ-opioid receptor agonist significantly decreased µ-opioid receptor-induced hypophagia in FD$_3$ neonatal layer-type chickens ($P \leq 0.05$) (Figure 7). Perhaps, the effect of µ-opioid receptor on appetite regulation is mediated via 5-HT$_2$c receptor.

In Experiment 8, administration of SB242084 (5-HT$_2$c receptor antagonist) at the level of 1.5 µg had no significant effect on cumulative food intake ($P \geq 0.05$). Likewise, the ICV injection of δ-opioid receptor agonist, DPDPE (40 pmol), significantly increased food consumption in FD$_3$ neonatal layer-type chickens ($P \leq 0.05$). Moreover, the injection of SB242084 + DPDPE had no significant effect on δ-opioid receptor-induced hyperphagia in FD$_3$ neonatal layer-type chickens ($P \geq 0.05$) (Figure 8). It seems 5-HT$_2$c receptors played no modulatory role in δ-opioid receptor-induced hyperphagia in neonatal layer-type chickens.

According to the data in Figure 9, the ICV injection of SB242084 (1.5 µg) had no significant effect on chicken's feeding behaviour ($P \geq 0.05$). In addition, the ICV injection of 30 nmol U-50488H (κ-opioid receptor agonist) significantly promoted food intake in comparison to control group ($P \leq$
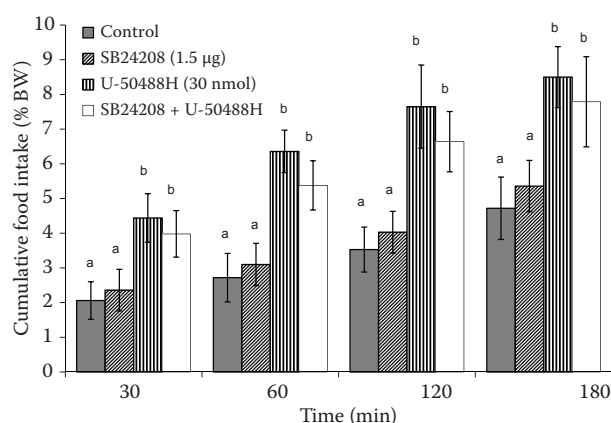
0.05). The co-injection of 5-HT$_2$c receptor antagonist (SB242084) with U-50488H was not able to alter the κ-opioid receptor-induced hyperphagia in FD$_3$ neonatal layer-type chickens ($P \geq 0.05$). Presumably, HT$_2$c receptors take no part in κ-opioid receptor induced hyperphagia.

DISCUSSION

To our knowledge, this is the first report on the effects of the interaction between opioidergic and 5-HTergic systems on food intake in FD$_3$ neonatal layer-type chicken. Results indicate that the ICV injection of µ-opioid receptor agonist decreases cumulative food intake in chicken until 3 h post-injection. The suppressive effect of µ-opioid receptor agonist lessening with time might be due to the half-life of the µ-opioid receptor ligand. It is well-documented that the opioidergic system impresses the orexigenic effect on feeding behaviour through nucleus accumbens (NAcc) and NTS µ-receptors in rodents (Fichna et al. 2007). µ-Opioid receptor mRNA expression increases after the ICV injection of morphine or DAMGO into hypothalamus in rat (Browning et al. 2006; Zheng et al. 2007). As observed, the effect of µ-opioid receptor is completely different in birds compared to mammals. These findings suggest that the central appetite regulatory mechanisms in chicken are presumably dissimilar from those in mammals. Studies sug-

gest that acute administration of opioids increases cumulative food intake in rat (Li et al. 2011). The orexigenic effect of μ-receptors is expressed via μ-receptors in NAcc and NTS in rodents (Fichna et al. 2007; Zheng et al. 2007).

To date, a few studies have been designed to investigate the effects of opioidergic system in poultry. Controversial results have been reported about the effect of μ-opioid receptors on food intake in meat-type chickens (McCormack and Denbow 1989; Bungo et al. 2004; Khan et al. 2009). In our study, DAMGO had greater effect at low (125 and 250 pmol) than at high doses (500 pmol) which was parallel to that in neonatal meat-type chicken (Bungo et al. 2004). Moreover, intramuscular (i.m.) or ICV injection of naloxone (opioid antagonist) diminished food intake in domestic fowl (Denbow and McCormack 1990). The observed biphasic effect of DAMGO suggests that at least two different mediators may be responsible for μ-opioid receptors dependent modification of food intake in birds (Raimondi et al. 2007).

Arcuate nucleus contains several neurotransmitters with complex interactions with each other. So, the outcomes of these interactions regulate energy expenditure and appetite (Shiraishi et al. 2008). Neuropeptide Y (NPY)/agouti related protein (AgRP) and pro-opiomelanocortin (POMC) and CART are the main sites which regulate appetite in the ARC (Zendehdel and Hassanpour 2014). Neural axons in the raphe nuclei reach to ARC and NAcc (Zendehdel et al. 2009, 2013a). It is suggested that μ-opioid receptors are involved in the orexigenic effect of NPY and AgRP neurons in the ARC (Barnes et al. 2006). The ICV injection of opioids or NPY increases food intake in rat and chicken. In particular, the orexigenic effect of NPY is mediated via μ-opioid receptors in meat-type chicken. Endogenous endorphins are ligands for μ-opioid receptors. In rat, NPY-producing neurons are synaptically linked with β-endorphin producing neurons in the ARC. So, it seems an interaction exists between the central opioidergic system and the NPY, however, the morphological link between the NPY and the opioidergic system has not been identified in poultry's hypothalamus (Dodo et al. 2005). In our study, μ-opioid receptor agonist showed opposite effect on appetite in layer-type neonatal chicken. In layers, but not in broilers, the food intake is mediated via AgRP, while NPY amplifies food intake both in broilers

and layers. Even though CNS is involved in appetite regulation in avian species, other sites might be involved as well (Shiraishi et al. 2008; Saneyasu et al. 2011; Bodnar 2012). Discrepancy between layer and broiler may be ascribed to genetic selection (Bungo et al. 2004). Genetic selection for growth in broilers altered their responsiveness to physiological appetite regulation mechanisms (Denbow 1994). To our knowledge, there is no previous research about the role of μ-opioid receptor agonists in ingestion in layer-type chicken. Our results provide evidence that μ-opioid receptor mediates hypophagia in layer-type chicks, which was different than in meat-type chicken. Further studies are needed to investigate the exact mechanism for this neural pathway in layers.

In Experiment 2, inverted U shaped dose response curve was observed for DPDPE. The ICV administration of 20 and 80 pmol of DPDPE had no significant effect, while a 40 pmol dose increased cumulative food intake in neonatal layer-type chicken. It is reported that the ICV injection of δ-opioid receptor agonist elevates food intake in neonatal meat-type chicken (Bungo et al. 2004; Yanagita et al. 2008; Khan et al. 2009) and rats (Levine 2006; Kaneko et al. 2012). The DPDPE-induced food intake diminished by the ICV injection of δ-opioid antagonist (Kaneko et al. 2012). In a former study, McCormack and Denbow (1989) observed that the ICV injection of met-enkephalin increased food intake in domestic fowl. Met-enkephalin is δ-opioidergic agonist which is found in dense fibre networks in the third ventricle in domestic fowl and sparrow (Maney and Wingfield 1998). In this regard, the ICV injection of δ-opioid antagonist (ICI-174,864) was not able to attenuate the NPY-induced feeding behaviour whereas deprivation-induced ingestion interacts with opioidergic system through the δ-opioid receptors in chicks (Dodo et al. 2005). McCormack and Denbow (1989) revealed that the ICV injection of β-endorphin stimulates food intake in layer-type chickens. β-Endorphin is an endogenous ligand for both μ- and δ-opioid receptors (Dodo et al. 2005) and stimulation of each receptor impresses its specific influence on food intake response in the CNS (Shiraishi et al. 2008). It seems that β-endorphin impresses the orexigenic effect via δ-opioid receptors in layers (Dodo et al. 2005; Webster et al. 2013). Recently, a new mechanism has been suggested for the role of central δ-opioid receptors in food intake regu-

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lation. In normal physiological condition, central prostaglandin D_2 (PGD_2) and NPY system play a deniable role in appetite and there is a neurological interaction between opioidergic and PGD_2–NPY system. The ICV injection of naltrindole (δ-opioid agonist) activates cyclooxygenase-2 (COX-2) which terminates to produce PGD_2. Then PGD_2 acts on its specific receptor, DP_1 (GPCR subtype), and stimulates NPY via DP_1-possessing NPY neurons in the CNS. So, it seems that the δ-opioid-induced orexigenic activity is mediated via the PGD_2–NPY system (Kaneko et al. 2012).

In Experiment 3, we determined the effect of κ-opioid receptors on cumulative food intake in FD_3 neonatal layer-type chickens. The ICV injection of κ-opioid receptor agonist increased food intake in FD_3 neonatal layer-type birds. It is reported that the ICV injection of κ-opioid receptor agonist (U-50488H) elicits hyperphagia in broiler (Bungo et al. 2004) and rat (Koch et al. 1992). The results of previous studies as well as of the current research suggest that κ-opioid receptor has orexigenic effect in mammals and birds. The ICV injection of κ-opioid receptor agonist to VTA or NAcc had no effect on feeding behaviour in rat (Le Merrer et al. 2009). Interestingly, NPY-induced food intake was not attenuated by κ-opioid receptor antagonist in rat (Jewett et al. 2001) whereas dynorphin (endogenous κ-opioid ligand) might be involved in the NPY-induced ingestion both in chicken and rat. It seems that different κ-opioid receptor agonists might act via other opioidergic receptors or pathways (Dodo et al. 2005).

There is an interaction among different opioidergic receptors. For instance, the ICV injection of μ- or δ- but not κ-receptor antagonist lessened DAMGO-induced food intake in rat. Maybe, κ-opioid receptors impress their effects via ARC proopiomelanocortin (POMC) neurons (Le Merrer et al. 2009). It is reported that the κ-opioid receptor activation inhibits adenylyl cyclase and CGRP release (Bodnar 2012).

Frequent researches on feeding behaviour have been done in rat models while just few studies have been designed in birds. The effect of the ICV injection of 5-HT in birds has rarely been studied. 5-HT exerts an inhibitory effect on feeding behaviour in avian. 5-HTergic receptors have different effect on feeding behaviour based on subtypes. Food intake weakens through 5-HT_2c receptors while 5-HT_{1A} receptor has no effect (Zendehdel et

al. 2013b, 2014), but 5-HT_{1A} receptors are crucial in central water intake regulation in rat (Villa et al. 2008). Presumably central appetite regulation mechanisms are somewhat different among animals. Martin-Ruiz et al. (2001) reported that a high density of 5-HT receptors has been identified in some parts of the CNS such as hypothalamus, prefrontal cortex (PFC) of pyramidal neurons.

The 5HTergic system plays a modulatory role in some effects of opioids such as antinociceptive effects of morphine. The co-administration of 5-HT receptor agonists along with μ-opioid agonist has been used for the treatment of pain (Li et al. 2011).

In our study, the co-administration of PCPA (cerebral 5-HT depletive) or SB242084 (5-HT_2c receptor antagonist) with DAMGO significantly blocked hypophagia induced by μ-opioid receptor in FD_3 neonatal layer-type chickens. Maybe, the effect of central μ-opioid receptors on food intake is mediated via the 5-HT_2c receptors in birds. Anatomically, endogenous opioids are present at high levels and affect the reward system in several of limbic regions such as the prefrontal cortex, amygdala, and NAcc. These nuclei receive 5-HTergic input from the dorsal raphe nucleus (DRN) (Kirby et al. 2011). Pharmacological studies revealed that 5-HT_2c receptors decrease food intake. The 5-HT_2c expression in POMC neurons might contribute to this effect. Furthermore, it is suggested that the hypophagic effect of 5-HTergic system is mediated via the melanocortin pathway (Nonogaki et al. 2008; Zendehdel et al. 2012a). The 5HT_2c receptors affect appetite regulation via several neurotransmitters (Zendehdel et al. 2013a, b, 2014). The melanocortin and β-endorphin neuropeptides are processed from POMC (Nonogaki et al. 2008). Anatomical reports demonstrate that opioid receptors (μ, δ, and κ) are present in DRN and the median raphe nuclei (MRN). Because of different receptor subtypes of both systems, the opioid-5-HT interaction is somewhat complex. For instance, the stimulation of μ- and δ-opioid receptors upregulates the 5-HT levels in DRN while the activation of κ-opioidergic receptors diminishes the 5-HT levels in DRN and MRN (Kirby et al. 2011). Also, 5-HT plays an important role in opioid biosynthesis (Di Benedetto et al. 2004). The direct mechanism of the interaction of μ-opioid and 5-HT_2c receptors has still been unclear (Kirby et al. 2011). Presumably the 5-HTergic and opioi-

dergic pathways of appetite regulation (based on different receptors) might cancel each other out (Di Benedetto et al. 2004).

Increasing evidences revealed that the 5-HT$_2$c receptor is located post-synaptically with a high degree of cellular heterogeneity with GABAergic neurons in the rat and human brain (Zendehdel et al. 2014). Perhaps, μ-opioid receptors act on the 5-HT$_2$c receptors by inhibition of GABA receptors which synapse on 5-HT cells in the raphe nuclei (Kirby et al. 2011). A blockade of 5-HT$_2$c receptors in the NAcc shell diminishes cocaine-induced locomotor activity (Navailles et al. 2004). The 5-HTergic system interacts with a variety of neurotransmitters on food intake. The inhibitory effect of dopamine on feeding behaviour is weakened via SB242084 (Zendehdel et al. 2014). Apparently, the effect of 5-HT$_2$c receptor on μ-opioid receptors is mediated via dopaminergic system (Navailles et al. 2004). It is described that opioid and 5-HTergic systems might be regulated via CART peptides in the CNS (Rothman et al. 2003). However, the involvement of other neurotransmitters cannot be excluded.

Based on the obtained results, the ICV injection of δ- and κ-opioid receptors agonists (DPDPE and U-50488H, respectively) amplified food intake and their effects were not attenuated by the 5-HT$_2$c receptor antagonist. Similar observations on hyperphagic effects of δ- and κ-opioid receptors have been reported in broiler and rat (Bungo et al. 2004; Yanagita et al. 2008; Khan et al. 2009; Kaneko et al. 2012). The ICV injection of NPY into the ventromedial hypothalamus (VMH) decreases the turnover of 5-HT (Shimizu and Bray 1989). Previous researches suggest that the κ-opioid receptor has orexigenic effect in mammals and birds (Le Merrer et al. 2009). The ICV injections of U-50488 and/or dynorphin into VTA and VMH stimulate food intake in rat. The mechanism of the κ-opioid receptor influence on feeding may consist in that the activated κ-opioid receptor might inhibit the corticotropin-releasing factor (CRF) which reinforces food intake (Bruijnzeel 2009). It seems there is no neurological interaction between the 5-HT-ergic system and the δ- and κ-opioid receptors in feeding behaviour of neonatal layer-type chickens. Perhaps, δ- and κ-opioid receptors induced hyperphagia is mediated by other neurological pathways in birds. It is reported that the κ-opioid receptor activation inhibits adenylyl cyclase and CGRP release (Bodnar 2012). To our knowledge, the regulatory role of the opioidergic system in food intake and its interaction with the 5-HTergic system is complex and the interaction(s) between these two systems might be regulated also by other neurotransmitters. Unfortunately, we have not detected any study concerning the direct effect of central 5-HT$_2$c receptors on the μ-opioid receptor induced hypophagia in birds so far. The novel findings presented herein could serve as a starting point in studying the interaction between central 5-HT$_2$c and μ-opioid receptors in neonatal layer-type chicken. We hope this study will trigger further research into the role of central opiates and 5-HTergic systems. So, merit studies are needed to clarify the direct neurological pathway(s).

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