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Central Opioidergic and Adrenergic systems Mediates Food Intake via α_1, α_2 and β_2 Receptors in Neonatal Layer-Type Chicken

Neda Nayebzadeh[1] · Bita Vazir[1] · Morteza Zendehdel[2] · Ahamd Asghari[3]

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Abstract
The aim of the current study was to determine possible interaction of central Opioidergic and Adrenergic systems on food intake regulation in neonatal layer-type chicken. In experiment 1, chicken ICV injected with control solution, DAMGO (μopioid receptors agonist, 125 pmol), parazosin (α_1 receptor antagonist, 10 nmol) and DAMGO + parazosin. In experiment 2, control solution, DAMGO (125 pmol), yohimbine (α_2 receptor antagonist, 13 nmol) and DAMGO + yohimbine were ICV injected. In experiment 3, FD_3 birds ICV injected with control solution, DAMGO (125 pmol), metoprolol (β_1 receptor antagonist, 24 nmol) and DAMGO + metoprolol. In experiment 4, FD_3 chicks received ICV injection of control solution, DAMGO (125 pmol), ICI 118,551 (β_2 receptor antagonist, 5 nmol) and DAMGO + ICI 118,551. Experiments 5–8 were similar to experiments 1–4, except chicken injected with DPDPE (δ opioid receptors agonist, 40 nmol) instead of DAMGO. Experiments 9–12 were similar to experiments 1–4, except chicken injected with U-50488H (κ opioid receptors agonist, 30 nmol) instead of DAMGO. Then, cumulative food intake was recorded at 30, 60 and 120 min after injection. According to the results, ICV injection of the DAMGO significantly decreased food intake while DPDPE and U-50488H significantly increased food intake in neonatal layer type chicken ($P < 0.05$). Co-injection of the DAMGO + ICI 118,551 decreased DAMGO-induced hypophagia ($P < 0.05$). Also, co-injection of the DPDPE + parazosin diminished hyperphagic effect of the DPDPE ($P < 0.05$). In addition, co-injection of the U-50488H + yohimbine diminished U-50488H-induced hyperphagia ($P < 0.05$). These results suggested there are interconnection between adrenergic and opioidergic systems on central food intake regulation which mediates via α_1, α_2 and β_2 receptors in neonatal layer-type chicken.

Keywords Opioidergic · Adrenergic · Food intake · Layer-type chicken

Introduction

Food intake regulation is a complex aspect which controls via diversity of central and peripheral neural pathways (Sharkey et al. 2014). In the central nervous system (CNS) it controls by interaction of the neurotransmitters in several brain nulei (Parker et al. 2014). The *locus coeruleus* (LC) is the major noradrenergic (NAergic) nucleus of the brain, giving Norepinephrine (NE, a catecholamine neurotransmitter) fibers innervating extensive areas (Tachibana et al. 2009). The NE receptors divided into α (α_1 and α_2) and β (β_1, β_2 and β_3) adrenergic receptors (Lei 2014). The brain NAergic system is included in many physiological functions such as appetite regulation and energy expenditure in both mammals and avian (Bungo et al. 1999). According to the reports micro injection of the NE into the paraventricular nucleus (PVN) increase food intake in broiler (Denbow and Sheppard 1993). Interestingly, ICV injection of the α_2 receptor agonist (clonidine) increased food intake and this effect was suppressed by yohimbine (α_2 receptor antagonist) not α_1 receptor antagonist (prazosin) (da Silva et al. 2017). Controversial reports exist for α-adrenergic receptors in avian. ICV injection of the clonidine increased food intake in broilers (Bungo et al. 1999) while NE had no effect on feeding behavior in layers (Denbow et al. 1981). β-adrenergic receptors are also, have role on metabolism and appetite. ICV injection

✉ Bita Vazir
bvazir@yahoo.com

[1] Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran 14155-6453, Iran

[3] Department of Clinical Science, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

of the isoproterenol (nonselective β adrenergic receptor agonist) and β_3 adrenergic receptor agonist decreased food intake in rats (Tsujii and Bray 1998). ICV injection of salbutamol (β_2 adrenergic receptor agonist) decreased cumulative food intake in rats (Kanzler et al. 2011). ICV administration of the isoproterenol weakened feeding and drinking behavior in broilers, respectively (Baghbanzadeh et al. 2010).

It is well documented that central appetite is not regulated by single neuropeptide and a wide distributed neural network interacts with other neurotransmitters on feeding and drinking behavior (Hassanpour et al. 2015). Opioids are inhibitory neurotransmitter and widely distributed in the CNS (Shojaei et al. 2015). Opioid receptors classified into 3 subtypes (μ, δ and κ) belonging to the G protein-coupled receptors (GPCRs) (Filizola and Devi 2013). They are responsible in several physiologic functions including pain, locomotion, neuroendocrine and reward (Kaneko et al. 2012). ICV injection of [D-Ala^2, NMe-Phe^4, Gly^5-ol]-enkephalin (DAMGO) and β-casomorphin (μ-opioid receptor agonists) induces hypophagia while [D-Pen^{2,5}]-enkephalin (DPDPE) (δ-opioid receptor agonist) exerts orexigenic effects in mammals (Kaneko et al. 2012). The ICV injection of the DAMGO (125 pmol) induces hypophagia while DPDPE (40 pmol) and U-50488H (30 nmol) has hyperphagic effect in neonatal layer and broiler chicks (Shojaei et al. 2015; Zendehdel et al. 2016). Based on the literature, interconnection exists between central Opioidergic and Adrenergic (ADergic) systems. It is reported activation of the opioid receptors inhibits the presynaptic release of the NA (Benyhe et al. 2015). Dimerization between μ-opioid and α_2 ADergic receptors can directly communicate with each other through the receptor complex (Benyhe et al. 2015). Administration of the opioid and α_2-adrenoceptor agonists into the spinal cord has synergistic analgesic effect (Trujillo et al. 2011). Administration of the ADergic agonists, particularly amphetamines enhances opioid-mediated reward behaviors (Root-Bernstein et al. 2018). It is reported p38 map kinase and β-arrestin 2 mediate neuronal functional regulates by interaction of μ and α_2 receptors (Tan et al. 2009). Despite the interaction observed between central opioidergic and ADergic systems, there is no report on interaction of these two systems on feeding behavior in mammalian and avian. Therefore, the aim of the current study was to determine possible interaction of central Opioidergic and ADergic systems on food intake regulation in neonatal layer-type chicken.

Materials and Methods

Animals

A total of 258 1-day-old layer chickens purchased from a local hatchery (Mahan Co., Iran). Birds were maintained in stabilizing electrically heated batteries at a temperature of 32 °C ± 1, kept at 40–50%relative humidity and 23:1 lighting/dark period (Olanrewaju et al. 2017). They were kept for 2 days as flocks and then birds randomly allocated into transferred into their individual cages. A commercial diet was offered during the study containing 21 percent crude protein and 2850 kcal/kg of metabolizable energy (Chineh Co., Iran) (Table 1). During the study all birds had ad libitum access to diet and fresh water. 3 h prior to the injections, birds were food deprived (FD_3) but had free access to water. ICV injections were done at 5 days of age. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, USA (publication No. 85-23, revised 1996) and the current laws of the Iranian government for animal care, and were approved by the Institutional Animal Ethics Committee of Faculty of Veterinary Medicine, University of Tehran.

Table 1 Ingredient and nutrient analysis of experimental diet

Ingredient	(%)	Nutrient analysis	
Corn	52.85	ME, kcal/g	2850
Soybean meal, 48% CP	31.57	Crude protein (%)	21
Wheat	5	Linoleic acid (%)	1.69
Gluten meal, 61% CP	2.50	Crude fiber (%)	3.55
Wheat bran	2.47	Calcium (%)	1
Di-calcium phosphate	1.92	Available phosphorus (%)	0. 5
Oyster shell	1.23	Sodium (%)	0.15
Soybean oil	1.00	Potassium (%)	0.96
Mineral premix	0.25	Chlorine (%)	0.17
Vitamin premix	0.25	Choline (%)	1.30
Sodium bicarbonate	0.21	Arginine (%)	1.14
Sodium chloride	0.20	Isoleucine (%)	0.73
Acidifier	0.15	Lysine (%)	1.21
DL-Methionine	0.10	Methionine (%)	0.49
Toxin binder	0.10	Methionine + cystine (%)	0.83
L-Lysine HCl	0.05	Threonine (%)	0.70
Vitamin D_3	0.1	Tryptophan (%)	0.20
Multi enzyme	0.05	Valine (%)	0.78

ME metabolisable energy, *CP* crude protein, per kg of diet, the mineral supplement contains 35.2 g manganese from $MnSO_4 \cdot H_2O$; 22 g iron from $FeSO_4 \bullet H_2O$; 35.2 g zinc from ZnO; 4.4 g copper from CuSO4•5H2O; 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g seleniumfrom Na_2SeO_3. The vitamin supplement contains 1.188 g of retinyl acetate, 0.033 g of dl-α-tocopheryl acetate, 8.84 g of tocopherol, 1.32 g of menadione, 0.88 g of thiamine, 2.64 g of riboflavin, 13.2 g of nicotinic acid, 4.4 g of pantothenic acid, 1.76 g of pyridoxin, 0.022 g of biotin, 0.36 g of folic acid, 1500 mg of choline chloride

Experimental Drugs

Drugs used include DAMGO (μ opioid receptors agonist), DPDPE (δ opioid receptors agonist, parazosin), U-50488H (κ opioid receptors agonist), parazosin (α_1 receptor antagonist), yohimbine (α_2 receptor antagonist), metoprolol (β_1 adrenergic receptor antagonist), ICI 118,551 (β_2 adrenergic receptor antagonist) and Evans blue were purchased from Sigma-Aldrich (USA) and tocris (UK) Co. All the drugs at first dissolved in absolute dimethyl sulfoxide (DMSO) then diluted with 0.85% saline containing Evans blue at a ratio of 1/250 (0.004% DMSO). The DMSO with this ratio does not have a cytotoxic effect (Blevins et al. 2002; Qi et al. 2008). DMSO/Saline containing Evans blue mixture used as control group.

ICV Injection Protocol

Birds randomly allocated into 8 experimental groups each having 4 sub-groups (n = 44). Prior to each experiment, the chicks were weighed and allocated into experimental groups based on their body weight (BW), thus, the average BW between treatment groups was as uniform as possible. The chicken was ICV injected once in each experiment using a microsyringe (Hamilton, Switzerland) without anesthesia using the Davis et al. (1979) and Furuse et al. (1997) method. Briefly, head of the chicken was held with an acrylic device in which the bill holder was 45° and the calvarium was parallel to the surface of table as explained by Van Tienhoven and Juhasz (1962). An orifice was made in a plate over the skull of right lateral ventricle. A microsyringe was inserted into the ventricle through the orifice in the plate and the tip of the needle perforated only 4 mm below the skin of the skull (Jonaidi and Noori 2012). All injections were done in a volume of 10 μL (Furuse et al. 1999). The control group received control solution (10 μL) (Furuse et al. 1999). This technique does not induce any physiological stress in neonatal chicks (Saito et al. 2005). At the end of the experiments, to recognize the accuracy of injection, the chicks were sacrificed by decapitation. Accuracy of placement of the injection in the ventricle was verified by the presence of Evans blue followed by slicing the frozen brain tissue. In each group, birds received injection, but just the data of those individuals where dye was present in their lateral ventricle were used for analysis (11 chickens per group). All experimental procedures were done from 0800 to 1330.

Feeding Experiments

In this study, 12 experiments were designed to determine the interconnection of the specific opioidergic receptors (μ, δ, and κ) and adrenergic receptors (α_1, α_2, β_1 and β_2) in FD_3 neonatal layer-type chicken. In experiment 1, chicken ICV injected with control solution, DAMGO (125 pmol), parazosin (10 nmol) and DAMGO + parazosin. In experiment 2, control solution, DAMGO (125 pmol), yohimbine (13 nmol) and DAMGO + yohimbine were ICV injected. In experiment 3, FD_3 birds ICV injected with control solution, DAMGO (125 pmol), metoprolol (24 nmol) and DAMGO + metoprolol. In experiment 4, FD_3 chicks received ICV injection of control solution, DAMGO (125 pmol), ICI 118,551 (5 nmol) and DAMGO + ICI 118,551. In experimet 5, the ICV injection to the birds were control solution, DPDPE (40 nmol), parazosin (10 nmol) and DPDPE + parazosin. In experiment 6, chicken ICV injected with control solution, DPDPE (40 nmol), yohimbine (13 nmol) and DPDPE + yohimbine. In experiment 7, control solution, DPDPE (40 nmol), metoprolol (24 nmol) and DPDPE + metoprolol was ICV injected. In experiment 8, FD_3 birds ICV injected with control solution, ICI 118,551 (5 nmol) and DPDPE + ICI 118,551. In experiment 9, chicken ICV injected with control solution, U-50488H (30 nmol), parazosin (10 nmol) and U-50488H + parazosin. In experiment 10, control solution, U-50488H (30 nmol), yohimbine (13 nmol) and U-50488H + yohimbine were ICV injected. In experiment 11, FD_3 birds ICV injected with control solution, U-50488H (30 nmol), metoprolol (24 nmol) and U-50488H + metoprolol. In experiment 12, FD_3 chicks received ICV injection of control solution, U-50488H (30 nmol), ICI 118,551 (5 nmol) and U-50488H + ICI 118,551. Immediately after the injection food provided to the birds and cumulative food intake (g) was measured at 30, 60 and 120 min after the injection. Food consumption (g) was calculated as percent of body weight (g/100 g BW) to minimize impact of body weight on the amount of food intake. These doses of drugs determined according to the previous studies (Bungo et al. 2005; Zendehdel and Hassanpour 2014; Zendehdel et al. 2017).

Statistical Analysis

Cumulative food intake was analyzed by repeated measure two-way analysis of variance (ANOVA) and is presented as the mean ± SEM. For treatments found to have an effect according to the ANOVA, mean values were compared with Bonferroni test. $P < 0.05$ were considered to indicate significant differences between the treatments.

Results

Interactions of the central opioidergic and adrenergic systems on food intake regulation in FD_3 neonatal chicks are shown in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12. In experiment 1, ICV injection of the DAMGO (125 pmol) significantly decreased food intake compared to control group ($P < 0.05$). ICV injection of the parazosin (10 nmol)

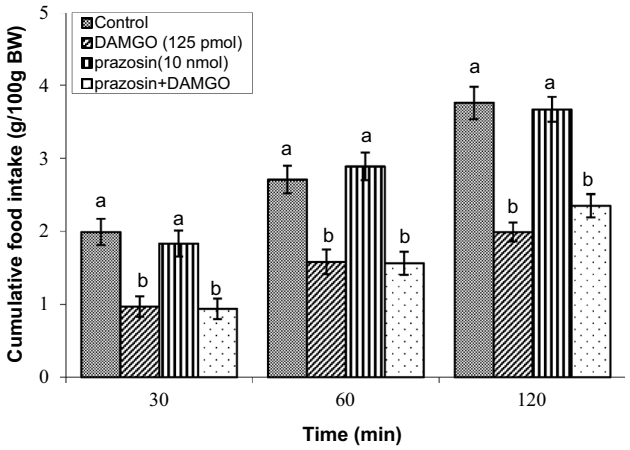

Fig. 1 Effect of ICV injection of parazosin (10 nmol), DAMGO (125 pmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DAMGO: μ opioid receptors agonist, parazosin: α_1 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

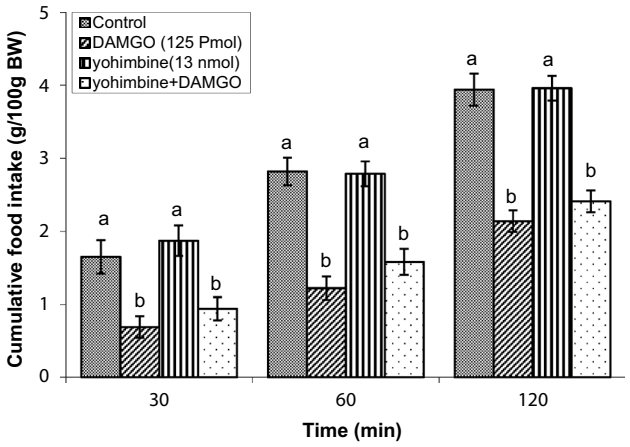

Fig. 2 Effect of ICV injection of yohimbine (13 nmol), DAMGO (125 pmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DAMGO: μ opioid receptors agonist, yohimbine: α_2 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

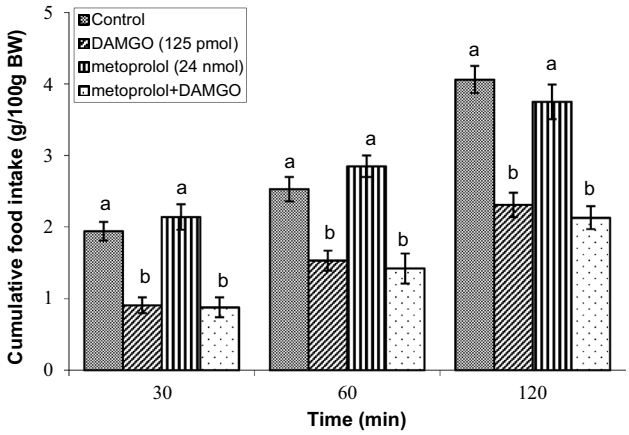

Fig. 3 Effect of ICV injection of metoprolol (24 nmol), DAMGO (125 pmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DAMGO: μ opioid receptors agonist, metoprolol: β_1 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

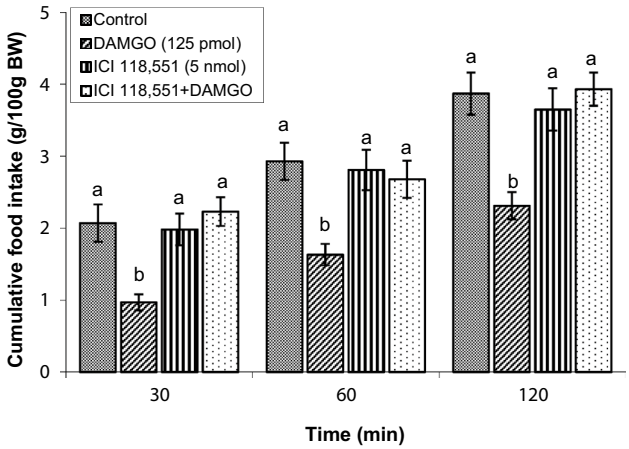

Fig. 4 Effect of ICV injection of ICI 118,551 (5 nmol), DAMGO (125 pmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DAMGO: μ opioid receptors agonist, ICI 118,551: β_2 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

had no significant effect on cumulative food intake compared to control group at 30, 60 and 120 min post-injection ($P > 0.05$). Co-injection of the DAMGO + parazosin had no effect on DAMGO-induced hypophagia in neonatal chicks ($P > 0.05$)(Treatment effect: F (3, 40) = 1538.43, $P < 0.001$; time effect: F (2, 80) = 3172.01, $P < 0.001$; treatment and time interaction: F (6, 80) = 18.43; $P < 0.001$) (Fig. 1).

In experiment 2, ICV injection of the DAMGO (125 pmol) significantly decreased cumulative food intake compared to control group ($P < 0.05$). ICV injection of the yohimbine (13 nmol) had no significant effect on food intake compared to control group ($P > 0.05$). Co-injection of the DAMGO + yohimbine had no significant effect on hypophagic effect of the DAMGO ($P > 0.05$) (Treatment effect: F (3, 40) = 2784.09, $P < 0.001$; time effect: F (2, 80) = 2835.06, $P < 0.001$; treatment and time interaction: F (6, 80) = 13.19; $P < 0.001$) (Fig. 2).

In experiment 3, hypophagia observed in chicken received ICV injection of the DAMGO (125 pmol). No significant effect on cumulative food intake observed in birds ICV injected with metoprolol (24 nmol) ($P > 0.05$). No significant effect observed on DAMGO-induced hypophagia by

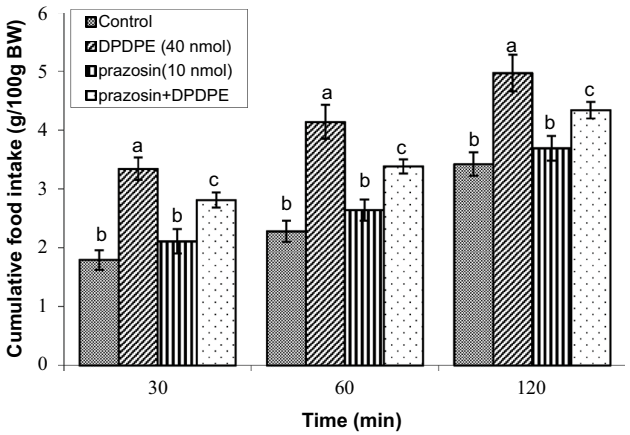

Fig. 5 Effect of ICV injection of parazosin (10 nmol), DPDPE (40 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DPDPE: δ opioid receptors agonist, parazosin: α_1 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments ($P < 0.05$)

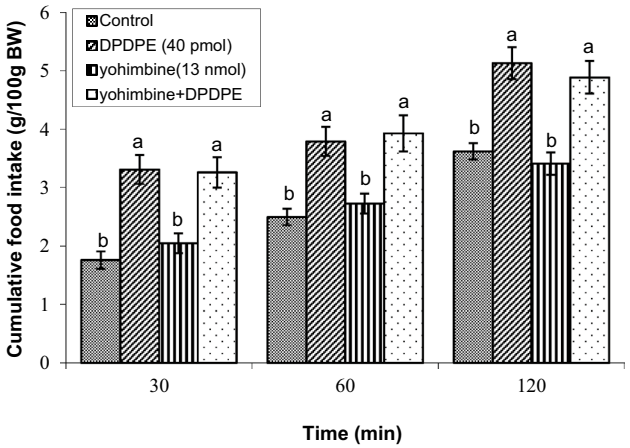

Fig. 6 Effect of ICV injection of yohimbine (13 nmol), DPDPE (40 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DPDPE: δ opioid receptors agonist, yohimbine: α_2 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

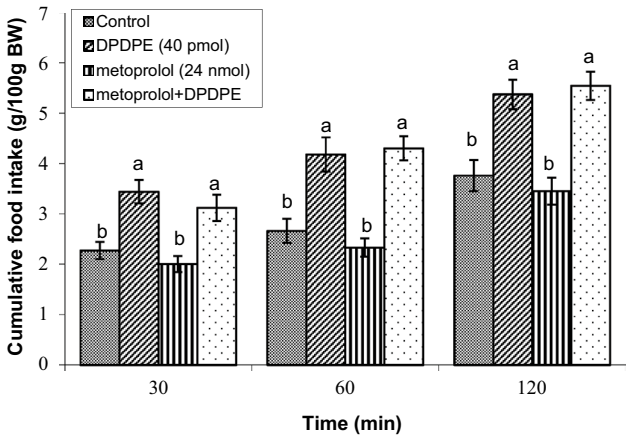

Fig. 7 Effect of ICV injection of metoprolol (24 nmol), DPDPE (40 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DPDPE: δ opioid receptors agonist, metoprolol: β_1 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

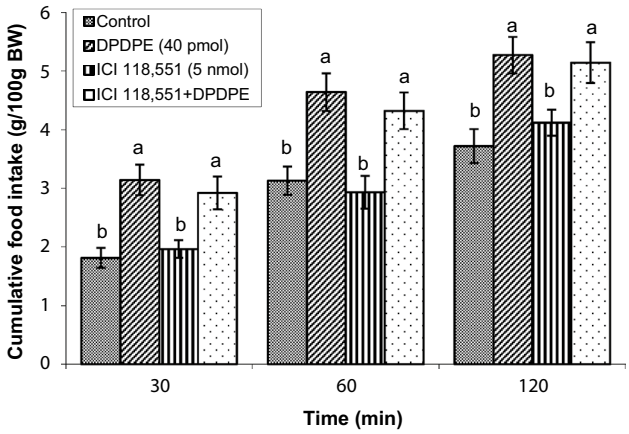

Fig. 8 Effect of ICV injection of ICI 118,551 (5 nmol), DPDPE (40 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). DPDPE: δ opioid receptors agonist, ICI 118,551: β_2 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

co-injection of the DAMGO + metoprolol ($P > 0.05$) (treatment effect: F (3, 40) = 3127.25, $P < 0.001$; time effect: F (2, 80) = 2874.81, $P < 0.001$; treatment and time interaction: F (6, 80) = 15.26; $P < 0.001$) (Fig. 3).

In experiment 4, ICV injection of the 125 pmol of the DAMGO significantly decreased cumulative food intake ($P < 0.05$). ICV injection of the ICI 118,551 (5 nmol) had no effect on food intake in neonatal layer ($P > 0.05$). Co-injection of the DAMGO + ICI 118,551 significantly decreased hypophagic effect of the DAMGO ($P < 0.05$) (Treatment effect: F (3, 40) = 963.52, $P < 0.001$; time effect: F (2, 80) = 3129.06, $P < 0.001$; treatment and time interaction: F (6, 80) = 26.71; $P < 0.001$) (Fig. 4).

In experiment 5, ICV injection of the DPDPE (40 nmol) significantly increased food intake compared to control group ($P < 0.05$). ICV injection of the parazosin (10 nmol) had no significant effect on cumulative food intake compared to control group ($P > 0.05$). Co-injection of the DPDPE + parazosin significantly increased hyperphagic effect of the DPDPE compared to the control group, however, this effect was weaker than sole DPDPE group ($P < 0.05$) (Treatment effect: F (3, 40) = 2126.14,

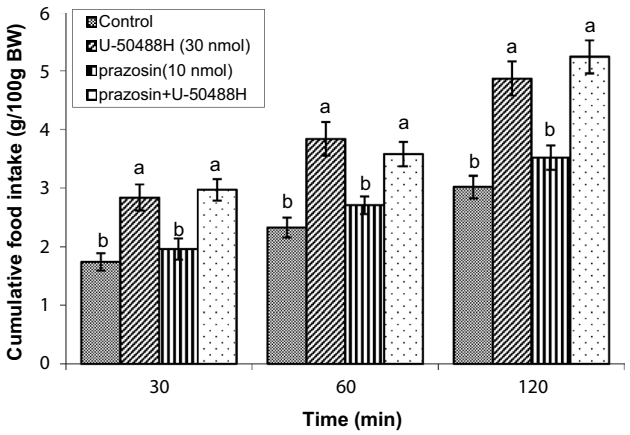

Fig. 9 Effect of ICV injection of parazosin (10 nmol), U-50488H (30 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). U-50488H: κ opioid receptors agonist, parazosin: α_1 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

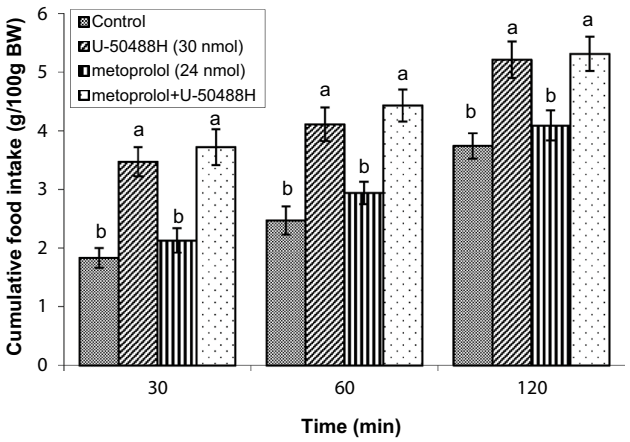

Fig. 11 Effect of ICV injection of metoprolol (24 nmol), U-50488H (30 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). U-50488H: κ opioid receptors agonist, metoprolol: β_1 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

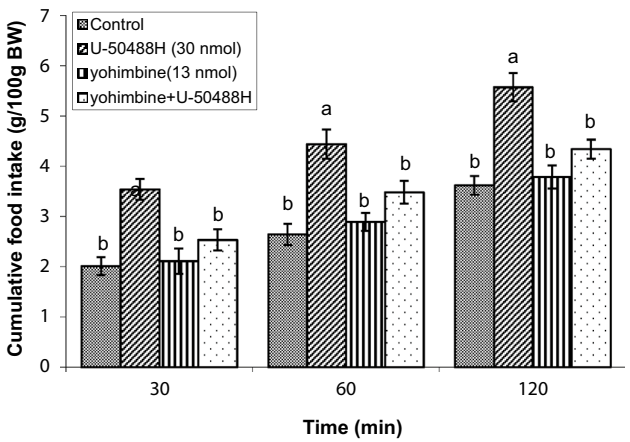

Fig. 10 Effect of ICV injection of yohimbine (13 nmol), U-50488H (30 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). U-50488H: κ opioid receptors agonist, yohimbine: α_2 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

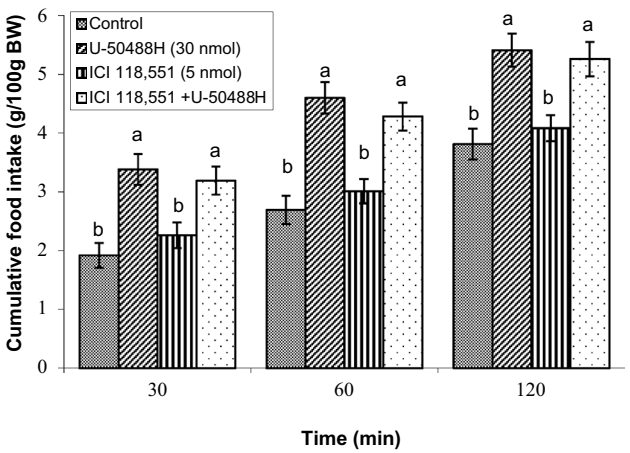

Fig. 12 Effect of ICV injection of ICI 118,551 (5 nmol), U-50488H (30 nmol) and their combination on percent of body weight cumulative food intake in neonatal layer type chicken (n = 44). U-50488H: κ opioid receptors agonist, ICI 118,551: β_2 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$)

$P < 0.001$; time effect: F (2, 80) = 3982.27, $P < 0.001$; treatment and time interaction: F (6, 80) = 32.59; $P < 0.001$) (Fig. 5).

In experiment 6, ICV injection of the DPDPE (40 nmol) significantly increased food consumption compared to control group at 30, 60 and 120 min post-injection ($P < 0.05$). ICV injection of the yohimbine (13 nmol) ad no significant effect on food intake compared to control group ($P > 0.05$). Co-injection of the DPDPE + yohimbine had no significant effect on hypophagic effect of the DPDPE ($P > 0.05$) (Treatment effect: F (3, 40) = 2836.06, $P < 0.001$; time effect: F (2, 80) = 2913.24, $P < 0.001$; treatment and time interaction: F (6, 80) = 13.09; $P < 0.001$) (Fig. 6).

In experiment 7, hyperphagic effect observed in chicken received ICV injection of the DPDPE (40 nmol). No significant effect on cumulative food intake observed in birds ICV injected with metoprolol (24 nmol) ($P > 0.05$). No significant effect observed on DAMGO-induced hypophagia by co-injection of the DPDPE + metoprolol ($P > 0.05$) (Treatment effect: F (3, 40) = 1807.09, $P < 0.001$; time effect: F (2, 80) = 3028.11, $P < 0.001$; treatment and time interaction: F (6, 80) = 17.06; $P < 0.001$) (Fig. 7).

In experiment 8, ICV injection of the 40 nmol of the DPDPE significantly amplified food intake compared to the control group ($P < 0.05$). ICV injection of the ICI 118,551 (5 nmol) had no effect on food intake in neonatal layer ($P > 0.05$). Co-injection of the DPDPE + ICI 118,551 had no effect on DPDPE-induced hyperphagia in neonatal layer ($P > 0.05$) (Treatment effect: F (3, 40) = 590.16, $P < 0.001$; time effect: F (2, 80) = 3021.72, $P < 0.001$; treatment and time interaction: F (6, 80) = 21.06; $P < 0.001$) (Fig. 8).

In experiment 9, U-50488H (30 nmol) significantly increased cumulative food consumption compared to control group ($P < 0.05$). ICV injection of the parazosin (10 nmol) had no significant effect on cumulative food intake compared to control group ($P > 0.05$). Co-injection of the U-50488H + parazosin had no effect on hyperphaigc effect of the U-50488H—($P > 0.05$) (Treatment effect: F (3, 40) = 2394.08 $P < 0.001$; time effect: F (2, 80) = 2814.71, $P < 0.001$; treatment and time interaction: F (6, 80) = 14.06; $P < 0.001$) (Fig. 9).

In experiment 10, ICV injection of the U-50488H (30 nmol) significantly amplified food intake compared to control group ($P < 0.05$). ICV injection of the yohimbine (13 nmol) ad no significant effect on food intake compared to control group ($P > 0.05$). Co-injection of the U-50488H + yohimbine had no significant effect on hypophagic effect of the U-50488H ($P > 0.05$) (Treatment effect: F (3, 40) = 3028.37, $P < 0.001$; time effect: F (2, 80) = 2439.07, $P < 0.001$; treatment and time interaction: F (6, 80) = 16.48; $P < 0.001$) (Fig. 10).

In experiment 11, hyperphagia observed in chicken received ICV injection of the U-50488H (30 nmol). No significant effect on cumulative food intake observed in birds ICV injected with metoprolol (24 nmol) ($P > 0.05$). Also, no significant effect observed on U-50488H (30 nmol)-induced hypophagia by co-injection of the U-50488H + metoprolol ($P > 0.05$) (Treatment effect: F (3, 40) = 3184.39, $P < 0.001$; time effect: F (2, 80) = 2619.17, $P < 0.001$; treatment and time interaction: F (6, 80) = 21.09; $P < 0.001$) (Fig. 11).

In experiment 12, ICV injection of the 30 nmol of the U-50488H significantly elevated cumulative food intake ($P < 0.05$). ICV injection of the ICI 118,551 (5 nmol) had no effect on food intake in neonatal layer ($P > 0.05$). Co-injection of the U-50488H + ICI 118,551 had no effect on hyperphagic effect of the U-50488H ($P > 0.05$) (Treatment effect: F (3, 40) = 3409.09, $P < 0.001$; time effect: F (2, 80) = 2927.45, $P < 0.001$; treatment and time interaction: F (6, 80) = 18.42; $P < 0.001$) (Fig. 12).

Discussion

The present study was designed to investigate the possible interconnection of the central Opioidergic and ADergic systems on food intake regulation in neonatal layer-type chicken. To the best of our knowledge, this is the first report on interconnection between Opioidergic and ADergic systems on food intake regulation in neonatal layer-type chicken. Based on the findings, ICV injection of the DAMGO significantly decreased food intake while increased by DPDPE and U-50488H in neonatal layer type chicken. In line with this result, Bungo et al. (2005) reported ICV injection of β-funaltrexamine (β-FNA: μ-opioid receptors antagonist) attenuated anorexic effect of the DAMGO in meat-type chicks. Opioid receptors constitute the most extensive (Feng et al. 2012) and play an important role in the ingestion of food in chicks. For example, ICV administration of the β-endorphin decreased food intake in broiler chicks (Yanagita et al. 2008). Anorexic effect of the endomorphin-2 into the chick brain mediates through μ-opioid receptors (Bungo et al. 2007). However, ICV injection of the DAMGO into the nucleus accumbens stimulated Feeding behavior in rats (Zheng et al. 2007). Similarly, ICV injection of the DPDPE stimulated food intake in neonatal broiler and mice (Kaneko et al. 2012). Comparative physiological studies suggested central mechanisms for award regulation pathways are dissimilar in mammalian and birds (Zendehdel and Hassanpour 2014). It seems there are also differences on food intake regulation between the broiler and layers (Hassanpour et al. 2015). Layer genetically selected for slow growth and body weight while broilers have higher feed consumption, basal metabolic rate and energy expenditure. Genetic selection altered chicken's brain neurological pathways associated with appetite and energy expenditure (Denbow 1994; Richards 2003).

According to the results, co-injection of the DAMGO + ICI 118,551 decreased DAMGO-induced hypophagia. Co-injection of the DPDPE + parazosin diminished hyperphagic effect of the DPDPE. Also, co-injection of the U-50488H + yohimbine diminished U-50488H-induced hyperphagia. Controversial reports exist on role of the ADergic receptors on feeding behavior in avian. ICV injection of the clonidine stimulated food intake in broiler (Bungo et al. 1999). ICV injection of the NE had no effect on food consumption in chicken (Denbow et al. 1981). In broilers, ICV injection of the norepinephrine into the Paraventricular (PVN) and ventromedial (VMH) hypothalamus increased while injection into the Reticularis superiorand pars dorsalis and Tractus occipitomesencephalicus decreased feed intake (Denbow 1999). Additionally, Baghbanzadeh and Hajinezhad (2010) reported food and water intake diminished by ICV injection of the β adrenergic receptor antagonists in broilers. ICV injection of ICI 118,551 (β_2 adrenergic receptor antagonists, 5 nMol) or SR 59230R (β_3 adrenergic receptor antagonists, 20 nMol) increased cumulative food intake in broilers (Zendehdel and Hasasnpour 2014). ICV injection of the nonselective β adrenergic receptor agonist (isoproterenol) decreased (Wellman 1992) where injection

of β_3 adrenergic receptor agonist increased food intake in rats (Tsujii and Bray 1998). Perhaps, adrenergic receptors have both stimulatory and inhibitory role on appetite. There is report for dimerization between μ-opioid and α-adrenergic receptors can directly communicate with each other via the receptor complex (Benyhe et al. 2015). Presynaptic κ-opioid interacts with the α_2 receptors on noradrenergic nerve terminals (Allgaier et al. 1989). Presynaptic opioid receptors inhibit NA release by κ-receptors in rabbit hippocampus (Crowley and Kash 2015). An interaction reported between the α_2 and adenosine receptor mechanisms in hippocampus and brain cortex which is similar to the α_2-opioid receptor interaction (Allgaier et al. 1989). It seems presynaptic receptors on the same axon terminal discriminate extracellular signals mutually influence signal transduction by a receptor interaction. Activation of the α adrenergic and μ-opioid receptors on the noradrenergic cell bodies in locus ceruleus increases K^+ conductance and leads to hyperpolarization. Both morphine and NA induce major inhibitory effects in brain neurons and peripherally by activating G protein-coupled-receptors (GPCRs). Also, Ca^{2+} channels are effector in opioid G proteins coupled receptors. In rat brain cortex, activation of presynaptic μ-opioid receptors decrease NA release via reduction of the Ca^{2+} influx and cAMP activation (Allgaier et al. 1989).

NPY-induced feeding has a closely related to the opioidergic system through the μ-opioid receptor in CNS in chicks (Dodo et al. 2005). In a study, Andrade et al. (2007) reported activation of the lateral parabrachial nucleus α_2 adrenergic receptor increase water and this effect locked by serotonergic, gabaergic and opioidergic system. Heterodimerization of opioidergic and ADergic receptors activate common signal transduction pathways from the original receptors (Ramanathan and Cryer 2011). Endogenous opioid decreases adrenal gland catecholamine release and this effect is reversed by naloxone administration suggested that modulation of the counter regulatory response to hypoglycemia occurs both centrally and peripherally. Also, blockade of adrenergic receptor inhibits antecedent hypoglycemia's ability to decrease the sympathoadrenal response to subsequent hypoglycemia (Ramanathan and Cryer 2011). ADergic and μ-opioid receptors bind to opioid and ADergic compounds which indicating these classes of receptors might evolved from a common predecessor (Root-Bernstein et al. 2018). Both ADergic and opioid compounds bind to μ-opioid receptors extracellular loop peptide. ADergic and opioids bind to extracellular loop peptides and to the intact μ-opioid receptors (Manglik et al. 2012). This low-affinity, combined opioid-adrenergic binding site would explain the antagonism of ADergic and opioid compounds for opioids receptors (Root-Bernstein et al. 2018). NAergic neurotoxins xylamine bind to opioid receptors and higher affinity of ADergic antagonists effectively compete with opioids for opioid receptor (Root-Bernstein et al. 2018). There are reports on opioid-adrenergic synergy by enhancement of opioid binding in the presence of ADergic agonists by the opioid and ADergic receptors (Rozenfeld and Devi 2011). Epinephrine and clonidine inhibit the tachyphylaxis caused by opiate analgesia while epinephrine but not propranolol or phentolamine, can reverse "acute tolerance" caused by repeated doses of morphine on guinea pig ileum (Chabot-Doré et al. 2015). In a previous study to determine the role of the β-ADergic and opioid receptors in antinociceptive effect of α,β-methylene-ATP at the supraspinal site, Fukui et al. (2001) reported ICV pretreatment with propranolol dose-dependently attenuated the antinociceptive effect α,β-methylene-ATP. ICV pretreatment with butoxamine and ICI-118,551, but not atenolol, attenuated the antinociception produced by α,β-methylene-ATP suggesting for antinociceptive effect of the β_2 receptors.

Numerous researches on central food intake regulation have done with rat models. It is known that central food intake regulation is dissimilar between mammals and birds (Zendehdel and Hassanpour 2014). Thus, it is logical to assume that regulatory mechanisms governing these processes in birds (Hassanpour et al. 2015). As seen, there was no previous report on interconnection of the ADergic and opioidergic receptors on food intake. So, authors were not able to compare our results with it. This information can be used as basic data on central feeding behavior in chicken. In conclusion, these results suggested there are interconnection between ADergic and opioidergic systems on central food intake regulation which mediates via α_1, α_2 and β_2 receptors in neonatal layer-type chicken.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed Consent This manuscript does not contain any studies with human subjects performed by any of the authors.

Research Involving Human and Animal Rights All experiments were executed according to the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal ethics committee.

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