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Original Article

Interconnection between Adrenergic and Dopaminergic Systems in Feeding Behavior in Neonatal Chicks

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Abstract

Central dopaminergic (DAergic) and adrenergic systems have a prominent role in appetite regulation; however, their interaction(s) have not been studied in neonatal layer chickens. Therefore, the current study aimed to determine the interaction of central DAergic and noradrenergic systems in food intake regulation in neonatal layer chickens. In the first experiment, chickens received the intracerebroventricular (ICV) injection of a control solution, prazosin (i.e., α_1 adrenergic receptor antagonist; 10 nmol), dopamine (DA; 40 nmol), and prazosin plus DA. The second to fifth experiments were similar to the first experiment except that the birds were injected with yohimbine (i.e., α_2 receptor antagonist; 13 nmol), metoprolol (i.e., β_1 adrenergic receptor antagonist; 24 nmol), ICI 118,551 (i.e., β_2 adrenergic receptor antagonist; 5 nmol), and SR59230R (i.e., β_3 adrenergic receptor antagonist; 20 nmol) instead of prazosin. In the sixth experiment, the chickens received ICV injection with the control solution and noradrenaline (NA; 75, 150, and 300 nmol). In the seventh experiment, the birds were injected with the control solution, SCH23390 (i.e., D_1 DAergic receptor antagonist; 5 nmol), NA (300 nmol), and SCH23390 plus NA In the eighth experiment, the control solution, AMI-193 (i.e., D_2 DAergic receptor antagonist; 5 nmol), NA (300 nmol), and AMI-193 plus NA were injected. Then, cumulative food intake was recorded at 30, 60, and 120 min after the injection. According to the obtained results, the ICV injection of DA (40 nmol) significantly decreased food intake in comparison to that reported for the control group ($P<0.05$). The co-injection of yohimbine plus DA significantly amplified DA-induced hypophagia in the neonatal chickens ($P<0.05$). In addition, the co-administration of ICI 118,551 plus DA significantly inhibited the hypophagic effect of DA in the neonatal chickens ($P<0.05$). Furthermore, NA (75, 150, and 300 nmol) significantly reduced food intake in a dose-dependent manner ($P<0.05$). The co-injection of SCH23390 plus NA decreased the hypophagic effect of NA in the neonatal chickens, compared to that reported for the control group ($P<0.05$). The co-injection of AMI-193 plus NA diminished NA-induced hypophagia, compared to that reported for the control group ($P<0.05$). The aforementioned results suggested that there is an interconnection between central DAergic and noradrenergic systems through α_2/β_2 adrenergic and D_1/D_2 DAergic receptors in food intake regulation in neonatal chicks.

Keywords: Adrenergic, Dopamine, Food intake, Layer chicken

Interconnexion entre les Systèmes Adrénergiques et Dopaminergiques dans le Comportement Alimentaire des Poussins Néonatals

Résumé: Les systèmes dopaminergiques (DAergic) et adrénergiques centraux ont un rôle de premier plan dans la régulation de l'appétit; cependant, leur(s) interaction(s) n'a (ont) pas été étudiée chez les poulets de couche néonatals. Par conséquent, la présente étude visait à déterminer l'interaction des systèmes DAergique et

noradrénergique centraux dans la régulation de la prise alimentaire chez les poulets de ponte néonatals. Dans la première expérience, les poulets ont reçu l'injection intracérébroventriculaire (ICV) d'une solution témoin, de la prazosine (c'est-à-dire, un antagoniste des récepteurs adrénergiques α_1; 10 nmol), de la dopamine (DA; 40 nmol) et de la prazosine plus DA. Les deuxième à cinquième expériences étaient similaires à la première expérience, sauf que les oiseaux ont reçu une injection de la yohimbine (c.-à-d. antagoniste du récepteur α_2; 13 nmol), du métoprolol (c.-à-d. antagoniste du récepteur adrénergique β_1; 24 nmol), ICI 118.551 (c.-à-d. β_2 antagoniste des récepteurs adrénergiques; 5 nmol) et SR59230R (c'est-à-dire antagoniste des récepteurs adrénergiques β_3; 20 nmol) au lieu de la prazosine. Dans la sixième expérience, les poulets ont reçu une injection d'ICV avec la solution témoin et de la noradrénaline (NA; 75, 150 et 300 nmol). Dans la septième expérience, les oiseaux ont reçu une injection de la solution de contrôle, SCH23390 (c.-à-d., Antagoniste du récepteur D_1 DAergic; 5 nmol), NA (300 nmol) et SCH23390 plus NA. Dans la huitième expérience, la solution de contrôle, AMI-193 (c'est-à-dire, un antagoniste du récepteur D_2 DAergique; 5 nmol), NA (300 nmol) et AMI-193 plus NA ont été injectés. Ensuite, la prise alimentaire cumulée a été enregistrée 30.60 et 120 minutes après l'injection. Selon les résultats obtenus, l'injection ICV de DA (40 nmol) a significativement diminué la prise alimentaire par rapport à celle rapportée pour le groupe témoin ($P < 0.05$). La co-injection de la yohimbine et de DA a considérablement amplifié l'hypophagie induite par la DA chez les poulets nouveau-nés ($P<0.05$). De plus, la co-administration d'ICI 118,551 plus DA a inhibé de manière significative l'effet hypophagique de la DA chez les poulets nouveau-nés ($P<0.05$). En outre, NA (75, 150 et 300 nmol) a réduit de manière significative la prise alimentaire en fonction de la dose ($P<0.05$). La co-injection de SCH23390 plus NA a diminué l'effet hypophagique de NA chez les poulets nouveau-nés, par rapport à celle rapportée pour le groupe témoin ($P<0.05$). La co-injection d'AMI-193 plus NA a diminué l'hypophagie induite par NA, par rapport à celle rapportée pour le groupe témoin ($P<0.05$). Les résultats susmentionnés suggèrent qu'il existe une interconnexion entre les systèmes DAergique et noradrénergique centraux via les récepteurs α_2/β_2 adrénergiques et D_1/D_2 DAergiques dans la régulation de la prise alimentaire chez les poussins nouveau-nés.
Mots-clés: Adrénergique, Dopamine, Prise alimentaire, Poulet en couches

1. Introduction

Appetite regulation is one of the complex aspects of animals. It modulates several parts of the brain cooperating with signals from the peripheral organs (Sharkey et al., 2014). In the central nervous system (CNS), appetite is controlled by diverse neurotransmitters via complex neurological pathways (Parker et al., 2014). Noradrenaline (NA) is a catecholamine neurotransmitter in the CNS (Tachibana et al., 2009). The NA has two major receptors, namely α adrenergic (i.e., α_1, and α_2) and β adrenergic (i.e., β_1, β_2, and β_3) receptors (Lei, 2014). The brain adrenergic system has a key role in appetite regulation and energy expenditure in mammals and avians (Bungo et al., 1999).

The intracerebroventricular (ICV) injection of NA into the paraventricular nucleus increases food intake in domestic fowl (Denbow and Sheppard, 1993). The ICV injection of NA or clonidine (i.e., α_2 receptor agonist) increases food intake and this effect is inhibited by α_2 receptor antagonist (i.e., yohimbine), not by α_1 receptor antagonist (i.e., prazosin) (Wellman et al., 1993). The ICV injection of clonidine increased food intake in broilers (Bungo et al., 1999). The ICV administration of NA had no effect on feeding behavior in layers (Denbow et al., 1981). The ICV injection of salbutamol (i.e., β_2 adrenergic receptor agonist) decreased cumulative food intake in rats (Kanzler et al., 2011). The ICV injection of isoproterenol (i.e., β_1 and β_2 adrenergic receptor agonists) decreased food and water intake in broilers, respectively (Baghbanzadeh et al., 2010).

Dopamine (DA) is a main catecholamine neurotransmitter in the brain and plays a crucial role in appetite regulation. Dopaminergic (DAergic) neurons expressed in different nucleus of the brain include the substantia nigra, ventral tegmental area (VTA), and hypothalamus. To date, at least, five distinct subtypes of DA receptors (i.e., D_1-D_5) have been identified (Cadet et al., 2010). The DA impresses its mediatory effects through at least five

distinct G protein-coupled receptor subtypes (Cadet et al., 2010). The D_1 and D_2 receptors are more abundant than other DA receptors in the brain DAergic system participating in many physiological functions, such as emotion, locomotor activity, cognition, and food intake (Madhavan et al., 2013).

Food intake decreased via D_1 and D_2 receptors in rats (Volkow et al., 2011). In addition, DA-induced hypophagia is mediated by D_1 receptors in chickens; however, other receptors (i.e., D_2-D_4) may have no role in appetite regulation (Zendehdel et al., 2017). It is well documented that central feeding behavior is not regulated via a single neuropeptide and a wide distributed neural network interacts with other neurotransmitters in feeding status (Irwin et al., 2008).

The ICV injection of the α_2 receptor antagonist has a crucial role in the treatment of Parkinson's disease (Chopin et al., 1999). In addition, the systemic injection of reboxetine (i.e., a selective NA reuptake inhibitor) increased the firing activity of DA neurons in the VTA (Guiard et al., 2008). Furthermore, the systemic administration of prazosin decreased the burst firing of DA neurons and supported the excitatory role of NA in the VTA (Guiard et al., 2008). Additionally, the firing rate and bursting activity of DA neurons increased by the injection of the selective α_2 receptor antagonist (i.e., idazoxan) (Guiard et al., 2008).

In a comparative physiological study, Cornil and Ball (2008) revealed that DA binds to α_2 receptors in birds and mammals. Based on the literature, there has been no information on the interaction between the DAergic and noradrenergic systems in food intake regulation in avians. Therefore, the current study aimed to determine the interaction of central DAergic and noradrenergic systems in food intake regulation in 3-hour food-deprived (FD_3) neonatal layer chickens.

2. Material and Methods

2.1. Animals

A total of 352 1-day-old layer chickens were purchased from a local hatchery (Mahan Co., Iran). The birds were maintained in stabilizing electrically heated batteries at a temperature of 32±1ºC, kept at 40-50% relative humidity, and 23:1 light/dark period (Olanrewaju et al., 2006). The chickens were kept for 2 days as flocks, and then the birds randomly were allocated into eight experiments and transferred into their cages. A commercial diet was offered during the study containing 21% crude protein and 2,850 kcal/kg of metabolizable energy (Chineh Co., Iran) (Table 1). During the study, all the birds had ad libitum access to diet and freshwater. Moreover, 3 h prior to the injections, the birds were food-deprived but with free access to water. The ICV injections were administered 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. In addition, animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee of Faculty of Veterinary Medicine, University of Tehran, Iran.

2.2. Experimental Drugs

The drugs used in this study included DA, NA, prazosin (10 nmol) (i.e., α_1 receptor antagonist), yohimbine (13 nmol) (i.e., α_2 receptor antagonist), metoprolol (24 nmol) (i.e., β_1 adrenergic receptor antagonist), ICI 118,551 (5 nmol) (i.e., β_2 adrenergic receptor antagonist), SR 59230R (2 nmol) (i.e., β_3 adrenergic receptor antagonist), SCH23390 (i.e., D_1 receptor antagonist; 5 nmol), AMI-193 (i.e., D_2 receptor antagonist; 5 nmol), NA, and Evans blue purchased from Sigma-Aldrich (USA) and Tocris (UK). All the drugs were firstly dissolved in absolute dimethyl sulfoxide (DMSO) and then diluted with 0.85 % saline containing Evans blue at a ratio of 1/250 (0.4% DMSO). The DMSO with this ratio does not have a cytotoxic effect (Blevins et al., 2002; Qi et al., 2008). The DMSO/Saline mixture containing Evans blue was used for the control group.

Table 1. Ingredient and nutrient analysis of experimental diet

Ingredient	(%)	Nutrient analysis	
Corn	52.85	ME (kcal/g)	2,850
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 hydrochloride	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 containing 35.2 g manganese from MnSO4·H2O, 22 g iron from FeSO4·H2O; 35.2 g zinc from ZnO, 4.4 g copper from CuSO4·5H2O, 0.68 g iodine from ethylene diamine dihydroiodide, and 0.12 g selenium from Na2SeO3; the vitamin supplement containing 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 pyridoxine, 0.022 g of biotin, 0.36 g of folic acid, and 1500 mg of choline chloride

2.3. ICV Injection Protocol

The birds were randomly allocated into eight experimental groups each one having four sub-groups (n=44). Prior to each experiment, the chicks were weighed and allocated into the experimental groups based on their body weight (BW); therefore, the average BW among the treatment groups was as uniform as possible. The chickens received ICV injection once in each experiment using a microsyringe (Hamilton, Switzerland) without anesthesia using the method of Davis et al. (1979) (Furuse et al., 1997). Briefly, the head of the chicken was held with an acrylic device in which the bill holder was 45º and the calvarium was parallel to the surface of the table as explained by Van Tienhoven and Juhasz (1962). An orifice was made in a plate over the skull of the 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 the injections were performed in a volume of 10 μL (Furuse et al., 1999). The control group received a control solution (i.e., DMSO/saline mixture containing Evans blue; 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 the injection, the chicks were sacrificed by decapitation. The 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, 11 birds received the injection; however, only the data of those in whom the dye was present in the lateral ventricle were used for analysis (9-11 chickens per group). All the experimental procedures were followed within 08:00 to 13:30.

2.4. Feeding Experiments

In this study, eight experiments were designed to determine the possible role of specific noradrenergic receptors (i.e., α_1, α_2, β_1, β_2, and β_3) in the hypophagic effect of DA on FD_3 neonatal broiler chickens. In the first experiment, the chickens received ICV injection

with the control solution, prazosin (10 nmol), DA (40 nmol), and prazosin plus DA. In the second experiment, the ICV injection of the control solution, yohimbine (13 nmol), DA (40 nmol), and their combination were administered. In the third experiment, the FD_3 birds received ICV injection with the control solution, metoprolol (24 nmol), and DA (40 nmol) and co-injection of metoprolol plus DA. In the fourth experiment, the FD_3 chicks received the ICV injection of the control solution, ICI 118,551 (5 nmol), and DA (40 nmol) and co-injection of ICI 118,551 plus DA. In the fifth experiment, the ICV injections into the birds were the control solution, SR 59230R (20 nmol), DA (40 nmol), and their combination. In the sixth experiment, the chickens received ICV injection with the control solution and NA (75, 150, and 300 nmol). In the seventh experiment, the birds were injected with the control solution, SCH23390 (i.e., D_1 receptor antagonist; 5 nmol), NA (300 nmol), and SCH23390 plus NA. In the eighth experiment, the control solution, AMI-193 (i.e., D_2 receptor antagonist; 5 nmol), NA (300 nmol), and AMI-193 plus NA were injected. Table 2 shows the illustration of the experimental procedures and treatments during the study. Immediately after the injection, food was provided to the birds and cumulative food intake (g) was measured at 30, 60, and 120 min after the injection. Food consumption was calculated at a gram of BW (g/100 g BW) to minimize the impact of BW on the amount of food intake. All doses of the drugs were determined according to a pilot study and previous studies (Zendehdel and Hassanpour, 2014; Zendehdel et al., 2017).

2.5. Statistical Analysis

Cumulative food intake was analyzed by repeated measure two-way analysis of variance (ANOVA) and is presented as mean±standard error of the mean. Regarding the treatments observed to have an effect according to the ANOVA, mean values were compared using the Bonferroni test. A p-value of less than 0.05 was considered to indicate significant differences between the treatments.

Table 2. Treatment procedures in the 1st to 8th experiments

1st Exp.	**ICV injection**
Treatment groups	
I	CS*
II	Prazosin (10 nmol)
III	Dopamine (40 nmol)
IV	Prazosin (10 nmol) + dopamine (40 nmol)

2nd Exp.	**ICV injection**
Treatment groups	
I	CS*
II	Yohimbine (13 nmol)
III	Dopamine (40 nmol)
IV	Yohimbine (13 nmol) + dopamine (40 nmol)

3rd Exp.	**ICV injection**
Treatment groups	
I	CS*
II	Metoprolol (24 nmol)
III	Dopamine (40 nmol)
IV	Metoprolol (24 nmol) + dopamine (40 nmol)

4th Exp.	ICV injection
Treatment groups	
I	CS*
II	ICI 118,551 (5 nmol)
III	Dopamine (40 nmol)
IV	ICI 118,551 (5 nmol) + dopamine (40 nmol)

5th Exp	ICV injection
Treatment groups	
I	CS*
II	SR 59230R (20 nmol)
III	Dopamine (40 nmol)
IV	SR 59230R (20 nmol) + dopamine (40 nmol)

6th Exp.	ICV injection
Treatment groups	
I	CS*
II	NA (75 nmol)
III	NA (150 nmol)
IV	NA (300 nmol)

7th Exp.	ICV injection
Treatment groups	
I	CS*
II	SCH23390 (5 nmol)
III	NA (300 nmol)
IV	SCH23390 (5 nmol) + NA (300 nmol)

8th Exp.	ICV injection
Treatment groups	
I	CS*
II	AMI-193 (5 nmol)
III	NA (300 nmol)
IV	AMI-193 (5 nmol) + NA (300 nmol)

ICV: Intracerebroventricular; CS: Control solution; prazosin: α_1 receptor antagonist; yohimbine: α_2 receptor antagonist; metoprolol: β_1 adrenergic receptor antagonist; ICI 118,551: β_2 adrenergic receptor antagonist; SR 59230R: β_3 adrenergic receptor antagonist; SCH23390: D_1 receptor antagonist; AMI-193: D_2 receptor antagonist; NA: Noradrenaline

3. Results

Figures 1-5 depict the effects and interactions of central DAergic and adrenergic systems in cumulative food intake in the FD_3 neonatal layers. In the first experiment, the ICV injection of prazosin (10 nmol) did not affect food intake ($P>0.05$); nevertheless, DA (40 nmol) significantly decreased food intake, compared to that reported for the control group ($P<0.05$). The co-injection of prazosin plus DA had no effect on DA-induced hypophagia in the neonatal chickens at 30, 60, and 120 min after the injection, compared to that reported for the control group ($P>0.05$; Figure 1).

In the second experiment, the ICV administration of yohimbine (13 nmol) had no effect on food intake (P>0.05); nonetheless, DA (40 nmol) had a hypophagic effect, compared to the control group (P<0.05). The co-injection of yohimbine plus DA amplified DA-induced hypophagia in neonatal chickens, compared to that reported for the control group (P<0.05; Figure 2).

In the third experiment, no significant difference was observed in food intake by the ICV injection of metoprolol (24 nmol). The ICV injection of DA (40 nmol) significantly decreased food intake in comparison to that reported for the control group (P<0.05). The co-injection of metoprolol plus DA had no effect on the hypophagic effect of DA (40 nmol), compared to that reported for the control group (P>0.05; Figure 3).

In the fourth experiment, the ICV injection of ICI 118,551 (5 nmol) did not affect food intake (P>0.05). The ICV injection of DA (40 nmol) significantly decreased food intake, compared to that reported for the control group (P<0.05). The co-administration of ICI 118,551 plus DA significantly inhibited DA-induced hypophagia in the neonatal chickens (P<0.05; Figure 4). In the fifth experiment, the ICV injection of SR 59230R (20 nmol) had no effect on food intake (P>0.05); however, DA (40 nmol) significantly reduced food intake, compared to that reported for the control group (P<0.05). In addition, the co-injection of SR 59230R plus DA had no effect on DA-induced hypophagia, compared to that reported for the control group (P>0.05; Figure 5).

In the sixth experiment, the ICV injection of NA (75, 150, and 300 nmol) in a dose-dependent manner decreased food intake, compared to that reported for the control group (P<0.05; Figure 6). In the seventh experiment, the ICV administration of SCH23390 (5 nmol) had no effect on food intake (P>0.05); nevertheless, NA (300 nmol) had a hypophagic effect, compared to the control group (P<0.05). The co-injection of SCH23390 plus NA decreased the hypophagic effect of NA in the neonatal chickens, compared to the control group (P<0.05; Figure 7).

In the eighth experiment, no significant difference was observed in food intake by the ICV injection of AMI-193 (5 nmol). The ICV injection of NA (300 nmol) significantly decreased food intake in comparison to that reported for the control group (P<0.05). The co-injection of AMI-193 plus NA diminished NA-induced hypophagia, compared to that reported for the control group (P<0.05; Figure 8).

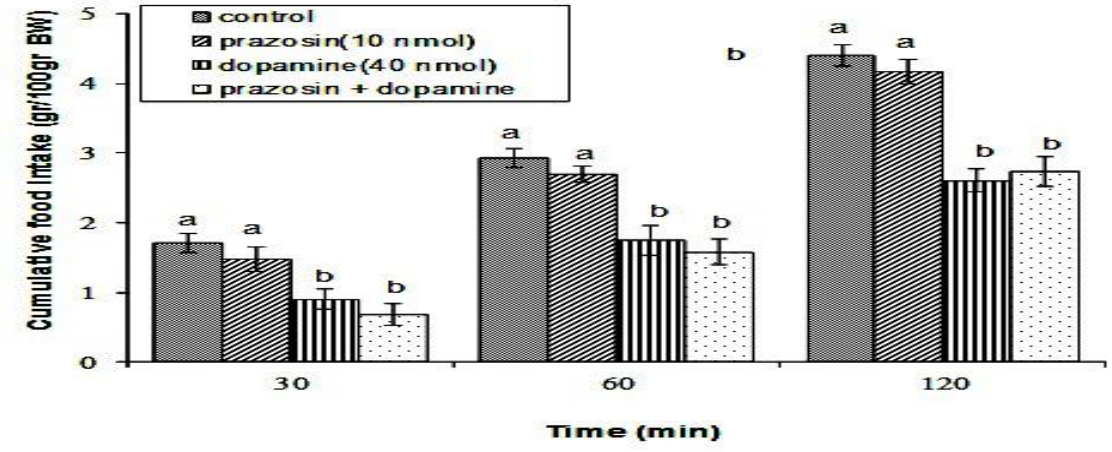

Figure 1. Effect of intracerebroventricular injection of prazosin (10 nmol), dopamine (40 nmol), and their combination on percentage of body weight (BW) cumulative food intake in neonatal layer chickens (n=44); prazosin: α_1 receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a and b) indicating significant differences between treatments (P<0.001)

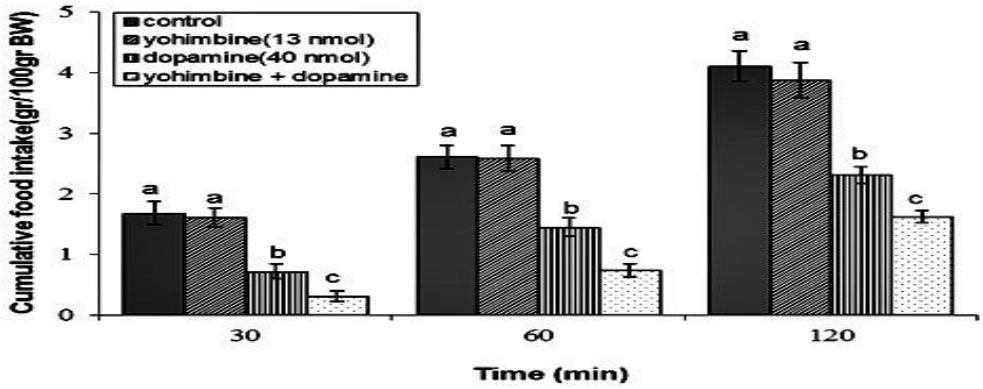

Figure 2. Effect of intracerebroventricular injection of yohimbine (13 nmol), dopamine (40 nmol), and their combination on percentage of body weight (BW) cumulative food intake in neonatal layer chickens (n=44); yohimbine: α_2 receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a, b, and c) indicating significant differences between treatments (P<0.001)

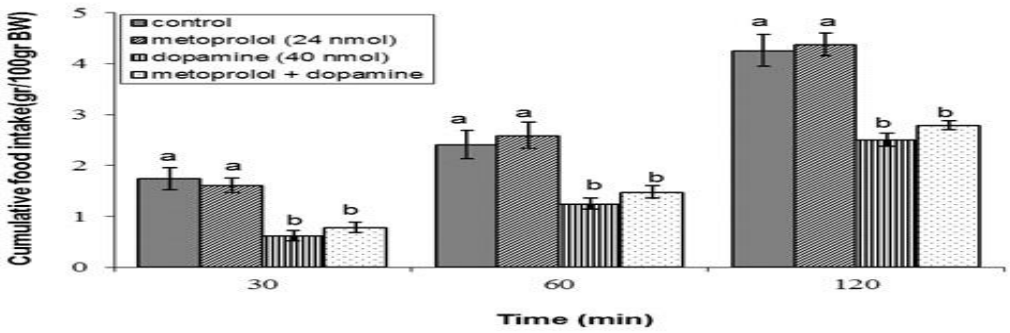

Figure 3. Effect of intracerebroventricular injection of metoprolol (24 nmol), dopamine (40 nmol), and their combination on percentage of body weight (BW) cumulative food intake in neonatal layer chickens (n=44); metoprolol: β_1 adrenergic receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a and b) indicating significant differences between treatments (P<0.001)

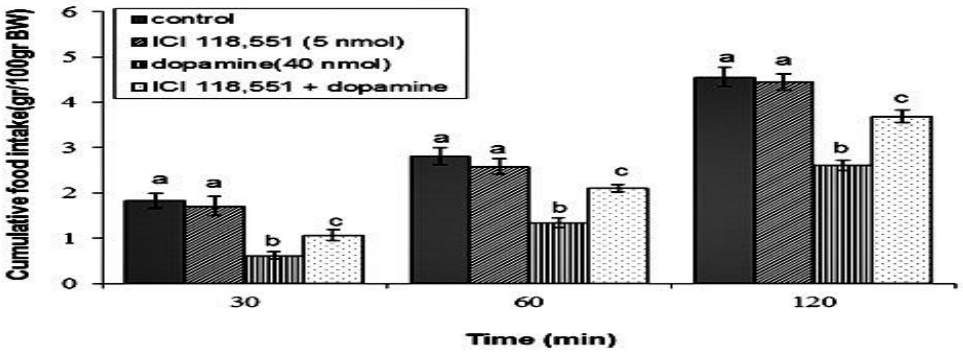

Figure 4. Effect of intracerebroventricular injection of ICI 118,551 (5 nmol), dopamine (40 nmol), and their combination on percentage of body weight (BW) cumulative food intake in neonatal layer chickens (n=44); ICI 118,551: β_2 adrenergic receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a, b, and c) indicating significant differences between treatments (P<0.001)

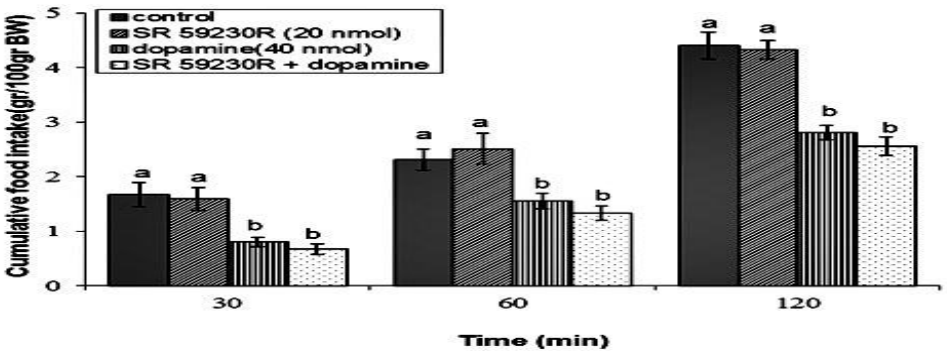

Figure 5. Effect of intracerebroventricular injection of SR 59230R (20 nmol), dopamine (40 nmol), and their combination on percentage of body weight (BW) cumulative food intake in neonatal layer chickens (n=44); SR 59230R: β_3 adrenergic receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a and b) indicating significant differences between treatments (P<0.001)

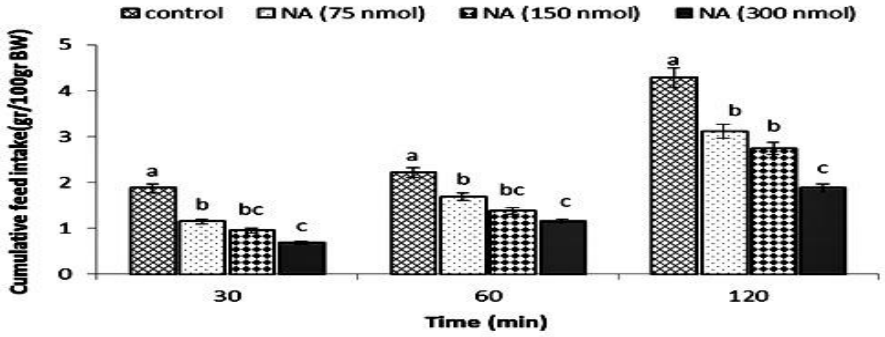

Figure 6. Effect of intracerebroventricular injection of noradrenaline (NA; 75, 150, and 300 nmol) on cumulative food intake in neonatal chickens (n=44); data expressed as mean±standard error of the mean; different letters (i.e., a, b, and c) indicating significant differences between treatments (P<0.001)

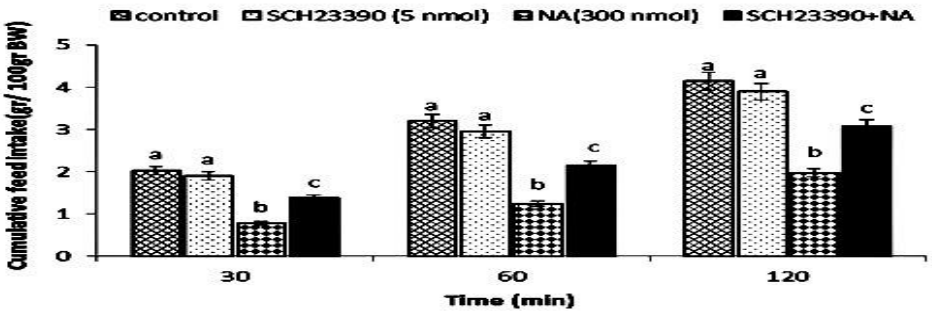

Figure 7. Effect of intracerebroventricular injection of SCH23390 (5 nmol), noradrenaline (NA; 300 nmol), and their combination on cumulative food intake in neonatal chickens (n=44); SCH23390: D_1 receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a, b, and c) indicating significant differences between treatments (P<0.001)

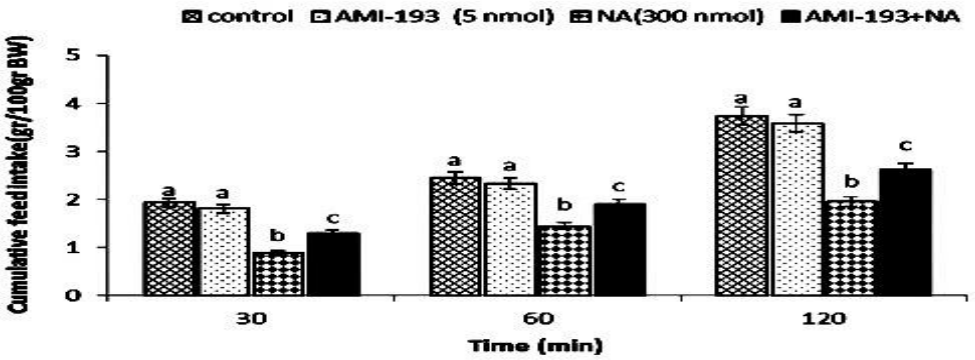

Figure 8. Effect of intracerebroventricular injection of AMI-193 (5 nmol), noradrenaline (NA; 300 nmol), and their combination on cumulative food intake in neonatal chickens (n=44); AMI-193: D_2 receptor antagonist; data expressed as mean±standard error of the mean; different letters (i.e., a, b, and c) indicating significant differences between treatments (P<0.001)

4. Discussion

To the best of our knowledge, this study has been the first report on the interaction of central DAergic and noradrenergic systems in food intake regulation in layer chickens. Based on the obtained results, the ICV injection of DA (40 nmol) decreased food intake in the FD_3 neonatal chickens. Recently, GhandForoushan et al. (2017) reported that the ICV injection of 40 nmol DA decreased food intake in FD_3 neonatal chickens. A daily decrease in cumulative food intake was reported using SKF 38393 (D_1) and apomorphine (D_2) receptor agonists in rats (Kuo, 2002). Dose-dependent response to food intake decrease was detected using SKF 38393 in both food-deprived and non-deprived rats (Terry and Katz, 1992).

The results of the current study are similar to the findings of our previous study in which the ICV injection of DA decreased food intake in broiler chickens (Zendehdel et al., 2014). The DA acts through its projections from the VTA into the nucleus accumbens (NAcc) and arcuate nucleus (Volkow et al., 2011). The DAergic neurons of the substantia nigra, pars compacta, VTA, and hypothalamus originate three main pathways, namely nigrostriatal, mesolimbocortical, and tuberoinfundibular, in the CNS (Cadet et al., 2010).

There have been controversial reports on the role of α-adrenergic receptors in feeding behavior in avians. The ICV injection of clonidine stimulated food intake in broilers (Bungo et al., 1999). Furthermore, Denbow et al. (1981) reported that the ICV injection of NE had no effect on food consumption in chickens. In broilers, the ICV injection of NA into the paraventricular and ventromedial nuclei increased feed intake while inhibited after ICV injection into the reticularis superior, pars dorsalis, and tractus occipitomesencephalicus (Denbow, 1999). The ICV injection of β adrenergic receptor antagonists decreased food and water intake in broilers (Baghbanzadeh et al., 2010).

Zendehdel and Hassanpour (2014) reported that the ICV injection of ICI 118,551 (i.e., β_2 adrenergic receptor antagonists; 5 nmol) or SR 59230R (i.e., β_3 adrenergic receptor antagonists; 20 nmol) increased cumulative food intake in broilers. It seems that adrenergic receptors have both stimulatory and

inhibitory roles in appetite regulation (Ferrari et al., 1991). The ICV injection of isoproterenol (i.e., nonselective β adrenergic receptor agonist) decreased food intake in rats (Wellman, 1992) where the injection of β_3 adrenergic receptor agonist showed an anorexigenic effect (Tsujii and Bray, 1998).

As observed, the co-injection of α_2 receptor antagonist (i.e., yohimbine; 13 nmol) plus DA amplified DA-induced hypophagia in the FD_3 neonatal chickens. The co-administration of the β_2 adrenergic receptor antagonist (i.e., ICI 118,551; 5 nmol) plus DA significantly inhibited DA-induced hypophagia in the FD_3 neonatal chickens. It was reported that the ICV injection of NA diminished feed intake in mammalians (Bungo et al., 1999).

There has been extensive literature on the promiscuous interaction between DA and adrenergic systems (Lin et al., 2008). The interaction between noradrenergic and DAergic neurons is mediated via α receptors (Nalepa et al., 2013). The ICV injection of prazosin (i.e., α_1 receptor antagonist) decreases the locomotor effect of acute amphetamine and cocaine (Drouin et al., 2002). As a result, DA-induced behavioral effects are primarily mediated via α receptors (Nalepa et al., 2013). Additionally, DA and adrenergic systems modulate theta and gamma oscillatory activity in the primary motor cortex via D_1, D_2, and α receptors (Ozkan et al., 2017).

Furthermore, the injection of DA increases intracellular Ca^{2+} release in pineal cells (Lei, 2014). However, intracellular Ca^{2+} is unaltered in response to D_1 receptor agonist (i.e., SKF-38393), D_2 receptor agonist (i.e., quinpirole), and D_1/D_2 receptor agonist (i.e., apomorphine) (Lei, 2014). The lack of DA receptor agonists in intracellular Ca^{2+} revealed that there is an alternative mechanism for the obtained results. For instance, prazosin (i.e., α_1 receptor antagonist) blocked DA-induced intracellular Ca^{2+} release revealing that intracellular Ca^{2+} release is mediated via α_1 adrenergic (Lei, 2014). The effects of DA blocked by α_1 receptor antagonist (i.e., prazosin) and α_2 receptor antagonist suggest the involvement of adrenergic system in DAergic receptors (Lei, 2014). In Parkinson's disease, due to the degeneration of the nigrostriatal DA pathway, α_2 receptor antagonists can increase the effect of the direct DA agonist apomorphine on circling behavior in rats indicating a facilitatory influence as a postsynaptic site to DA neurons (Chopin et al., 1999).

Despite the fact that direct mechanism(s) for the interaction between DAergic and adrenergic receptors is not fully determined, it was reported that D_1 receptors coupled to adenylyl cyclase, increasing cyclic adenosine monophosphate and activating protein kinase A (Ozkan et al., 2017). This phenomenon modulates voltage-dependent Na^+ channels, activates L-type Ca^{2+} channels, and attenuates a slowly inactivating outward rectifying K^+ current (Ozkan et al., 2017). The DA-induced membrane hyperpolarisation is mediated through adrenergic receptors (Yang et al., 2014).

Moreover, the interconnection of DA in adrenergic receptors in the entorhinal cortex produces membrane depolarisation via the inhibition of inward rectifier K^+ channels and enhances the frequency of miniature inhibitory postsynaptic potential and spontaneous inhibitory postsynaptic potential (Yang et al., 2014). The interaction between adrenergic and DA receptors acts as G-protein or second messenger systems (Ozkan et al., 2017). In addition, the interconnection was revealed between DAergic and β-adrenergic receptors. In patients with heart failure, the DA-induced inotropic effect is mediated via β adrenergic receptors (Lei, 2014).

The DA binds to α_2 receptors in quails and rats (Cornil and Ball, 2008). In the rodent brain, α_2 receptors receive DAergic inputs in conjunction or not with noradrenergic inputs, including the NAcc, amygdala, hypothalamic nuclei, and locus coeruleus (Cornil and Ball, 2008). The DA is released in these regions in response to stimuli, such as stress. Furthermore, because DA is a part of the synthesis

pathway of NE, it would be expected that noradrenergic terminals would always express DA (Cornil and Ball, 2008). The α_2 receptors expressed in the cell bodies and axons of mesoprefrontal DAergic neurons provide a morphological basis to the vast functional evidence that nerve-terminal α_2 receptors control DAergic activity and DA release in the prefrontal cortex (Castelli et al., 2016).

Although an axo-axonic relationship between DAergic and noradrenergic terminals remains to be recognized, extracellular NA may diffuse trans-synaptically to access through volume transmission and α_2 receptors in DA terminals (Fuxe et al., 2015). The local application of the selective α_2 receptor antagonist (i.e., idazoxan) reduced the suppressant effect on VTA DA neurons (Guiard et al., 2008). Idazoxan has a 1000-fold lower affinity for D_2 receptors than for α_2 receptor in addition to the stimulation of D_2 receptors in the rat cortex. The DA inhibits the DA neuron activity by α_2 receptors (Castelli et al., 2016). The role of α_2 receptors in the direct regulation of DA neurons remained debatable, especially in the rat brain in which the affinity of DA to α_2 receptors is lower than NE (Castelli et al., 2016).

In conclusion, DAergic and adrenergic systems are widely expressed in the CNS, with their distribution and expression levels largely mirroring the density of innervating fibers. Both the aforementioned systems are required for proper operation and cannot independently act without affecting the other system (Xing et al., 2016). High NE and DA levels cause the recruitment of α, β, and excessive D_1 receptor activation during abnormal conditions, such as stress, weakening neuronal firing, and prefrontal functioning (Xing et al., 2016).

Based on the literature, there has been no report on the interaction of these systems in food intake regulation in mammals and rodents. Therefore, the current study has been the first report in this regard and the obtained results suggested that there is an interaction between central DAergic and noradrenergic systems through α_2 and β_2 adrenergic and D_1 and D_2 DAergic receptors in food intake regulation in neonatal chicks. In addition to previous studies carried out on central food intake regulation in poultry, the obtained results of the present study revealed that the interaction between DAergic and adrenergic systems regulates appetite in chicks. There has been scarce information on the interaction of neurotransmitters in feeding behavior in avians. The obtained results of the present study can be used as base information for further studies. Furthermore, it is required to perform merit studies to determine cellular and molecular mechanism(s) involved in the interaction of DAergic and adrenergic systems.

Authors' Contribution

Study concept and design: M. Z.
Acquisition of data: F. Z.
Analysis and interpretation of data: N. P.
Drafting of the manuscript: N. P.
Critical revision of the manuscript for important intellectual content: N. P.
Statistical analysis: A. A.
Administrative, technical, and material support: M. Z.

Ethics

There is no informed consent in this study. This manuscript does not contain any studies with human subjects performed by any of the authors. All the experiments were carried out according to the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee of Science and Research Branch, Islamic Azad University, Tehran, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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