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Original Article

Impact of the Central Histaminergic and Melanocortin Systems on Leptin-Induced Hypophagia in Neonatal Layer Chicken

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Abstract

The present study aimed to assess the probable impact of the central histaminergic and melanocortin systems on leptin-induced hypophagia in neonatal layer chickens. In experiment 1, the chickens received intracerebroventricular (ICV) injections of the control solution, 250 nmol of α-FMH, 10 µg of leptin, and α-FMH+leptin. Experimental groups 2-8 were injected the same as experiment 1. Nonetheless, the chickens in experiments 2-8 received ICV injections of 300 nmol of chlorpheniramine (H_1 receptor antagonist), 82 nmol of famotidine (H_2 receptor antagonist), 300 nmol of thioperamide (H_3 receptor antagonist), 0.5 nmol of SHU9119 (M_3/M_4 receptors antagonist), 0.5 nmol of MCL0020 (M_4 receptor antagonist), 30 µg of astressin-B (CRF_1/CRF_2 receptors antagonist), and 30 µg of astressin2-B (CRF_2 receptor antagonist), instead of α-FMH, respectively. Food was provided for the birds immediately following the injection, and 30, 60, and 120 min after the injection, cumulative food intake (g) was measured. The findings pointed out that the ICV injection of leptin diminished food intake in neonatal chickens (P<0.05). The co-administration of M_3/M_4 receptor antagonist+leptin significantly decreased the hypophagic effect of leptin (P<0.05). A significant decrease was also detected in the hypophagic effect of leptin following the co-administration of the M_4 receptor antagonist and leptin (P<0.05). Moreover, the co-injection of the antagonists of CRF_1/CRF_2 receptors and leptin significantly mitigated the hypophagic effect of leptin (P<0.05). The co-injection of CRF_2 receptor antagonist and leptin led to a decrease in the hypophagic effect of leptin. As evidenced by the results of the current study the hypophagic effect of leptin is mediated by the receptors of H_1, H_3, M_3/M_4, and CRF_1/CRF_2 in neonatal layer chicken.

Keywords: Histaminergic, Melanocortin, Leptin, Food intake, Neonatal layer chicken

1. Introduction

There exist some differences between avian and mammalian species in terms of central regulation of food intake due to the regulatory impact of neurotransmitters and their receptors, apart from the relevant nucleus (1). Leptin is known as a peptide hormone secreted from the white adipose tissue, with blood levels being correlated with body fat mass (2). This hormone can cross the blood-brain barrier (3) and exert an effect on diverse hypothalamic areas, including the amygdala, nucleus tractus solitaries, and arcuate nucleus (ARC) (4) Leptin has a role to play in the regulation of feeding and satiety so that food intake diminished after the intracerebroventricular (ICV)

injection of leptin at the doses of 2.5, 5, and 10 µg in broiler chickens (2).

Central histaminergic neurons are located in the tuberomammillary nucleus (TMN) with axon projects branched to various brain areas (5). Brain histamine is of great importance in determining feeding behavior. Consequently, histamine administration through the ICV route reduced food intake, while food intake was elevated under the influence of chlorpheniramine, as an antagonist of H_1 receptor, and alpha-fluoromethylhistidine (α-FMH), as a selective inhibitor of the histamine-synthesizing enzyme histidine decarboxylase (6). Different physiological functions are mediated by the melanocortin system, namely grooming, thermoregulation, learning, and the regulation of energy balance. Among melanocortin receptors (MC1R-MC5R), MC3R and MC4R have been recognized to be crucial for central appetite regulation in the ARC, ventromedial hypothalamus, and periventricular nucleus (PVN) (7). The previously conducted studies have indicated that the ICV injection of the agonists of MC_3R/MC_4R receptors could reduce food intake in broiler chickens (8).

Some studies in the past decades assessed the central and peripheral systems responsible for the regulation of appetite in avians (9). The investigations that compared physiology pointed to some variations in the pathways of food intake regulation among mammals and avian species (10). Central leptin, histamine, and melanocortin systems were revealed to have interactions. Furthermore, previous investigations applied the histaminergic system for the leptin-induced suppression of food intake. Nonetheless, this influence is suggested to be indirect due to the lack of leptin receptor expression in the TMN (11). Some authors reported that the anorectic effect of leptin was amplified by the H_1 receptors (12).

Leptin is the link between the peripheral energy stores and proopiomelanocortin (POMC), signaling activity in the hypothalamus. It is worth noting that all mammalian hypothalamic POMC neurons do not express leptin receptors, indicating that a leptin-unrelated melanocortin signaling system may also exist. The diacetyl-α-MSH is acetylated to α-MSH, as a more active melanocortin, and the latter reaction is facilitated by leptin (13). Despite the interaction among leptin, histamine, and melanocortin, there is no report concerning their possible interactions in terms of food intake regulation in birds. In light of the aforementioned issues, the present study aimed to assess the impact of the central histaminergic and melanocortin systems on leptin-induced hypophagia in neonatal layer chickens.

2. Materials and Methods

2.1. Animals

The present study was performed on 352 one-day-old layer chickens (Hy-Line), which were purchased from a local hatchery (Morghak Co., Iran). Birds were kept in stabilizing electrically-heated batteries at the temperature, relative humidity, and lighting/dark cycle of 32±1ºC, 40%-50%, and 23:1, respectively (14). The subjects were kept at the mentioned conditions for 2 days as flocks, followed by random allocation and transferring to individual cages. A commercial diet was provided with 21% crude protein and 2850 kcal/kg metabolizable energy during the study (Chineh Co., Iran) (Table 1). Birds had free access to food and fresh water. The subjects were food-deprived 3 h before injections; nonetheless, they still had free access to water. The ICV injections were performed at the age of 5 days.

2.2. Experimental Medications

The administered medications were leptin, α-FMH, chlorpheniramine, famotidine (i.e., H_2 receptor antagonist), thioperamide (i.e., H_3 receptor antagonist), SHU9119 (i.e., M_3/M_4 receptors antagonist), MCL0020 (i.e., M_4 receptor antagonist), astressin-B (i.e., CRF_1/CRF_2 receptors antagonist), astressin2-B (i.e., CRF_2 receptor antagonist), and Evans blue. All the medications were bought from Sigma-Aldrich (USA) and Tocris Co. (UK) and dissolved in absolute

dimethyl sulfoxide (DMSO). Following that, the medicines were diluted using 0.85% saline containing Evans blue at a ratio of 1/250 (0.4% DMSO). No cytotoxic effect was observed for DMSO at this ratio (15, 16). The DMSO/saline mixture that had Evans blue was utilized for the control group.

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: metabolizable 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 $FeSO_4 \cdot H_2O$; 35.2 g zinc from ZnO; 4.4 g copper from $CuSO_4 \cdot 5H_2O$; 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g selenium from 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 pyridoxine, 0.022 g of biotin, 0.36 g *of folic acid, 1500 mg of* choline chloride.

2.3. Intracerebroventricular Injections

The subjects were randomly assigned to nine experimental groups with four sub-groups (n=44). The birds were weighed and allocated to test groups based on their body weight (BW) since the mean BW of diverse treatment groups was the same. The ICV injections were performed once for each group by a microsyringe (Hamilton, Switzerland) without anesthesia according to the techniques of Davis, Masuoka (17) and Furuse, Matsumoto (18). In brief, the chicken head was held applying an acrylic device with the bill holder at 45º. Calvarium was in a parallel position relative to table surface as described by Van Tienhoven and Juhasz (19). Thereafter, an orifice was made in a plate over the skull of the right lateral ventricle, through which a microsyringe was inserted. The needle tip perforated 4 mm under the skull skin (20), and the volume of all injections was 10 µL (21).

The animals in the control group received 10 µL of control solution (21). It is noteworthy that the mentioned method does not cause physiological stress for newly hatched chickens (22). To identify the accuracy of injection, decapitation was carried out at the end of the experiments. The accuracy of the injection site in the ventricle was confirmed based on the presence of Evans blue and the slicing of frozen brain tissues. All birds in each intervention group received injections. Nevertheless, only the data of 11 birds in each group, which had dye in their lateral ventricle were analyzed. All test procedures were performed during 8-13:30.

2.4. Feeding Experiments

In experiment 1, the birds received a control solution, 250 nmol of α-FMH, 10 µg of leptin, as well as a co-injection of α-FMH and leptin. The chickens in

experiment 2 received a control solution, 300 nmol of chlorpheniramine, 10 µg of leptin, and chlorpheniramine+leptin. Injections in experiment 3 were control solution, 82 nmol of famotidine, 10 µg of leptin, in addition to famotidine+leptin. The birds in experiment 4 received a control solution, 300 nmol of thioperamide, 10 µg of leptin, and thioperamide+leptin. In experiment 5, the control solution, 0.5 nmol of SHU9119, 10 µg of leptin, and SHU9119+leptin was injected. In experiment 6, the subjects were administered a control solution, 0.5 nmol of MCL0020, 10 µg of leptin, and MCL0020+leptin. The birds in experiment 7 received ICV injections of control solution, 30 µg of astressin-B, 10 µg of leptin, and astressin-B+leptin. In experiment 8, chickens received the control solution, 30 µg of astressin2-B, 10 µg of leptin, and astressin2-B+leptin. Immediately following the injections, the birds were fed, and cumulative food intake (g) was measured 30, 60, and 120 min after the injection. Food consumption (g) was calculated as the percent of body weight (g/100g BW) to minimize the influence of body weight on food intake. The latter doses of medications were determined based on the previous investigations (2, 8, 23, 24).

2.5. Statistical Analysis

In the current study, eight experimental groups were designed. Each test group entailed four subgroups (I-IV). Only one injection was performed in each group. Cumulative food intake stated as a percent of body weight was analyzed for each intervention group using two-way repeated-measures analysis of variance (ANOVA) by the following model:

$Y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$, with $\varepsilon_{ijk} \sim N(0, \sigma 2)$

where Y_{ijk} represents the value of individual observation for valuables, μ is the grand mean, α_j denotes the treatment effect for the time, β_k refers to the treatment effect for the medications, $(\alpha\beta)_{jk}$ denotes the effect of time×medicine interaction, and ε_{ijk} signifies an error. All the data were statistically analyzed in SPSS software (version 16) (IBM, Chicago, Il., USA). The means were compared by the Tukey test (P<0.05), and descriptive statistics are presented as mean±SEM (standard error of the mean).

3. Results

In experiment 1, hypophagia was observed after the ICV injection of 10 µg leptin, compared to the control group (P<0.05). Cumulative food intake was not affected by the injection of 250 nmol of α-FMH, in comparison with the control group (P>0.05). Moreover, the hypophagic effect of leptin significantly diminished due to the co-administration of α-FMH+leptin (P<0.05; Figure 1).

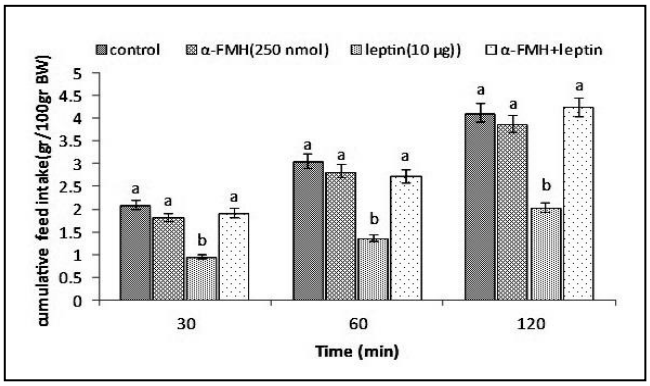

Figure 1. Effects of intracerebroventricular injection of control solution, α-FMH (250 nmol), and leptin (10 µg), as well as the co-injection of the α-FMH+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. α-FMH: alpha-Fluoromethylhistidine. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences between treatments at each time (P<0.05).

In experiment 2, hypophagia was detected following the ICV injection of 10 µg of leptin, in comparison with the control group (P<0.05). Chlorpheniramine injection through the ICV route (300 nmol) did not influence cumulative food intake, compared to the control group (P>0.05). The co-injection of chlorpheniramine and leptin resulted in a significant decline in the hypophagic effect of leptin (P<0.05; Figure 2).

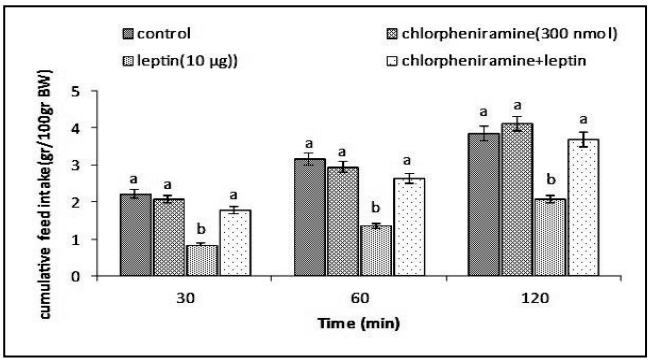

Figure 2. Effects of intracerebroventricular injection of control solution, chlorpheniramine (300 nmol), and leptin (10 µg), as well as the co-injection of the chlorpheniramine+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. Chlorpheniramine: H_1 receptor antagonist. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences between treatments at each time (P<0.05).

In experiment 3, the ICV injection of 10 µg of leptin significantly reduced food intake, in comparison with the control group (P<0.05). The ICV injection of 82 nmol famotidine did not affect cumulative food intake in this group, compared to the control group (P>0.05). Furthermore, co-injecting famotidine and leptin exerted no impact on hypophagia due to the existence of leptin in chickens (P>0.05; Figure 3).

In experiment 4, 10 µg of leptin was injected through the ICV route, causing the hypophagic effect, as compared to the control group (P<0.05). No significant alteration was observed in the cumulative food intake of this group following thioperamide injection (300 nmol) (P>0.05). However, the co-injection of thioperamide and leptin resulted in a significant reduction in the hypophagic effect of leptin (P<0.05; Figure 4).

In experiment 5, hypophagia was reported following the ICV injection of 10 µg leptin (P<0.05). It was observed that the ICV injection of 0.5 nmol SHU9119 did not influence food intake, compared to the control group (P>0.05). The administration of SHU9119 and leptin together caused a significant reduction in the hypophagic effect of leptin (P<0.05; Figure 5).

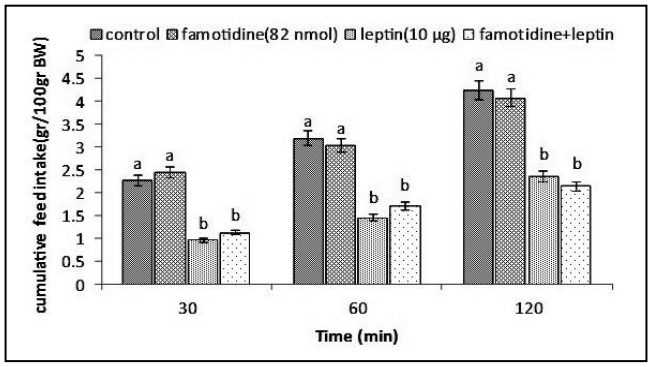

Figure 3. Effects of intracerebroventricular injection of control solution, famotidine (82 nmol), and leptin (10 µg), as well as the co-injection of the famotidine+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. Famotidine: H_2 receptor antagonist. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences between treatments at each time (P<0.05).

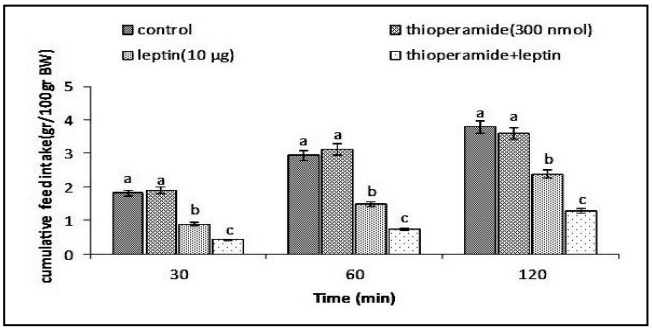

Figure 4. Effects of intracerebroventricular injection of control solution, thioperamide (300 nmol), leptin (10 µg), as well as the co-injection of the thioperamide + leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. Thioperamide: H_3 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time (P<0.05).

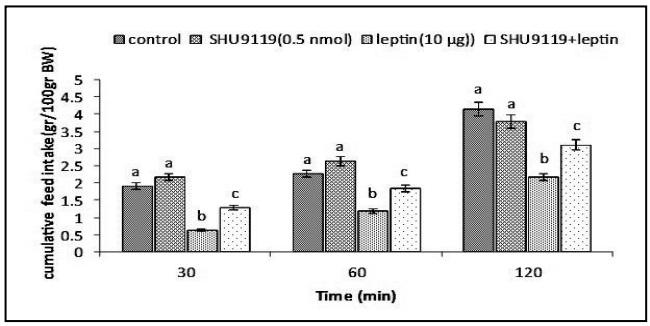

Figure 5. Effects of intracerebroventricular injection of control solution, SHU9119 (0.5 nmol), and leptin (10 µg), as well as the co-injection of the SHU9119+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. SHU9119: M_3/M_4 receptors antagonist. Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time (P<0.05).

In experiment 6, the ICV injection of leptin (10 µg) led to hypophagia, in comparison with the control group (P<0.05). The injection of 0.5 nmol MCL0020 did not affect food intake in this group, compared to the control group (P>0.05). The leptin-induced hypophagia decreased significantly after the injection of MCL0020 and leptin together (P<0.05; Figure 6).

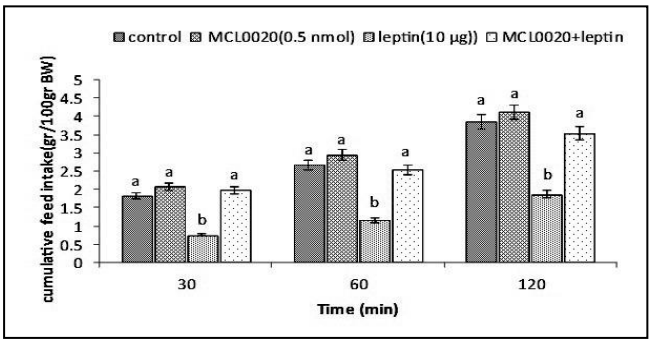

Figure 6. Effects of intracerebroventricular injection of control solution, MCL0020 (0.5 nmol), and leptin (10 µg), as well as the co-injection of the MCL0020+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. MCL0020: M_4 receptor antagonist. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences between treatments at each time (P< 0.05).

In experiment 7, food intake significantly diminished due to leptin ICV injection (P<0.05). Nonetheless, food intake did not change significantly after the injection of 30 µg astressin-B (P>0.05). The effect of leptin-induced hypophagic declined significantly after the co-administration of astressin-B+leptin (P<0.05; Figure 7).

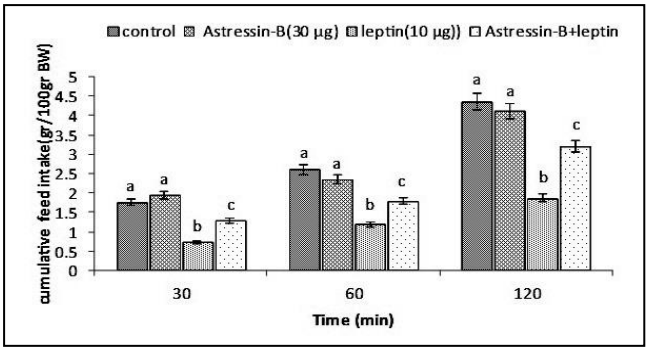

Figure 7. Effects of intracerebroventricular injection of control solution, astressin-B (30 µg), and leptin (10 µg), as well as the co-injection of the astressin-B+leptin on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. Astressin-B: CRF_1/CRF_2 receptors antagonist. Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time (P< 0.05).

In birds in experiment 8, leptin ICV injection caused a significant decrease in their food intake, compared to the subjects in the control group (P<0.05). The injection of astressin-2B (30 µg) through the ICV route imposed no significant impact on food intake, in comparison with the control group (P>0.05). The injection of astressin-2B and leptin together significantly reduced the hypophagic effect of leptin (P<0.05; Figure 8).

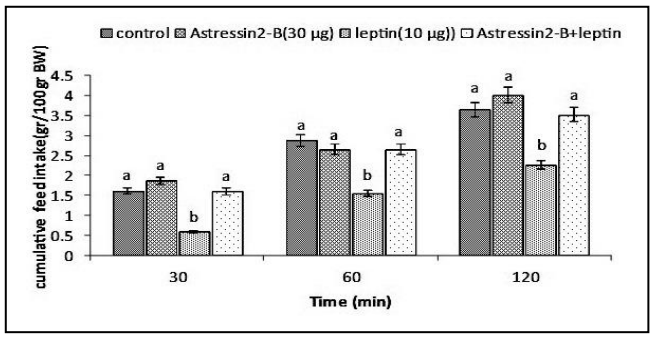

Figure 8. Effects of intracerebroventricular injection of control solution, astressin2-B (CRF_2 receptor antagonist; 30 µg), and leptin (10 µg), as well as the co-injection of the astressin2-B+leptin (10 µg) on cumulative food intake (gr/100gr BW) in neonatal chicks. Control: normal saline. Astressin2-B: CRF_2 receptor antagonist. Data are expressed as mean±SEM. Control: normal saline. Different letters (a and b) indicate significant differences between treatments at each time (P< 0.05).

4. Discussion

A review of the literature revealed that the present investigation is the first study on the role of the central glutamatergic system in the hypophagic effect of melatonin in neonatal broiler chickens. As evidenced by the obtained results, the injection of leptin through the ICV route diminished food intake in neonatal chickens. Leptin, which is secreted by the adipose tissue, can pass across the blood-brain barrier and bind its receptor to the central nervous system (25). Consistent with the results of the current study, it has been reported that the ICV injection of leptin may reduce food intake in broilers and Leghorns (22). Nevertheless, in a study by Furuse, Matsumoto (18), food consumption was not affected by the ICV injection of mouse leptin in neonatal Single-comb White Leghorn chickens. The genetic factors of animals, age, and the source of leptin might contribute

to the mentioned varieties. The gene that encodes leptin in chicken can be cloned (26). Although the types of leptin produced by chickens and rodents are different, they can be highly similar with 95% identical amino acids (27).

There is a dearth of information concerning the effect of leptin on CNS in birds. Leptin in birds triggers the downstream signaling pathways through its receptor. Nonetheless, the highest rate of the expression of their mRNA in distinct species takes place in the liver or brain (28). The sensitivity of broilers to peripheral leptin is lower than that of layer chickens (10). Food consumption, basal metabolism, and energy expenditure are all higher in broilers, which could be attributed to the genetic alterations in the mechanisms responsible for controlling food intake. Broilers are believed to be less sensitive to the anorexigenic signals from peripheral tissues (10).

According to the findings of the current study, the co-administration of the antagonists of H_1 receptors and leptin reduced leptin-induced hypophagia. Moreover, the hypophagic effect of leptin declined as the result of co-injecting an antagonist of H_2 receptors with leptin. Hypothalamic neuronal histamine, along with its H_1 receptor, plays a role in the formation of the leptin-signaling pathway in the brain. They were demonstrated to influence food intake and uncoupling protein mRNA expression, leading to the regulation of body weight and adiposity in mice with diabetes (db/db) or diet-induced obesity (29).

It was observed that α-FMH had the potential to inhibit food intake decrease induced by leptin in fasting rats. Leptin reduced the histamine content of the hypothalamus. On the other hand, it augments the ratio of tele-methylhistamine and histamine, signifying that leptin has the capacity to diminish the metabolism of HA. In addition, α-FMH suppressed Corticotropin-releasing hormone (CRH) expression both at the basic condition and following induction by leptin. On the contrary, it stimulated Neuropeptide Y (NPY) expression in fasting rats. In conclusion, histamine contributes to the inhibition of food intake, which is caused by leptin. Histamine can be a mediator and directly activates and/or alters the histaminergic system metabolism. The histaminergic system might play a role permissively (30).

The findings of the present study revealed that the co-administration of M_3/M_4 receptor antagonists and leptin caused a significant decline in leptin-induced hypophagia. The co-injection of an antagonist of the M_4 receptor and leptin significantly diminished the hypophagic effect of leptin. The co-administration of the antagonists of CRF_1/CRF_2 receptors+leptin led to a decline in the hypophagic effect of leptin. The injection of CRF_2 receptor antagonist and leptin reduced the hypophagic effect imposed by leptin. It has been suggested that the MC_4 receptor is of great importance for leptin signaling in rodents (29). The results of the current study were in agreement with the latter report. This receptor is known to be among the main pathways of leptin signaling in the hypothalamus (31).

Agouti protein may be over-expressed ectopically in Ay/a obese mice, resulting in higher food intake and body weight due to the antagonistic impact of MC_4 receptor that causes notable obesity in the abdomen region and leptin-resistant diabetes (32). Although leptin injection through the ICV route diminished food intake in wild-type mice, it did not happen in MC_4 receptor knockout obese or Ay/a obese mice (29). The mentioned results demonstrated that the MC_4 receptors were involved in the regulation of leptin-induced hypophagia.

Leptin and the MC_4 receptor can severely elevate adiposity, regardless of the type of diet consumed (33). In general, it is believed that MC_4 and H_1 receptors are involved in the regulation of energy homeostasis in the brain pathways downstream from the leptin action site. Nonetheless, there is a paucity of data about the relationship between neuronal histamine H_1 and MC_4 receptors (29). Leptin has the potential to stimulate α-MSH, as well as M_3 and M_4 receptor agonists. Moreover, it can inhibit Agouti-related peptide (AgRP), in addition to M_3 and M_4 receptor antagonists (34). In

line with the findings of the present research and the study by Morimoto, Yamamoto (35), it was reported that the CRH remarkably mediates the hypophagic effect of leptin (36). The blockade of histamine synthesis by α-FMH inhibits CRH gene expression, which is stimulated by leptin in the PVN, demonstrated that the influence of leptin results from interaction with the histaminergic neurons (30).

Histaminergic fibers project to diverse discrete areas, such as the PVN, in which the leptin receptors are expressed (30). Some authors have suggested that leptin might stimulate CRH directly in the PVN. Furthermore, the inhibition of CRH expression due to histaminergic system blockade by α-FMH demonstrates that the activation of the neuronal histamine system is needed (permissive effect) for CRH stimulation by leptin (30). The findings of the current study pointed out that H_1, H_3, M_3/M_4, and CRF_1/CRF_2 receptors mediate the hypophagic effect of leptin in neonatal layer chickens. It is recommended that further studies clarify the cellular and molecular signaling pathways that underlie the interactions among leptin, melanocortin, histamine systems, and food intake in neonatal chickens.

Authors' Contribution

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

Ethics

All the procedures approved by the ethics committee of the Islamic Azad University, Tehran, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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