BMC Veterinary Research

RESEARCH

Open Access

Involvement of central opioid and melanocortin receptors in spexin-induced hypophagia following intracerebroventricular injection in neonatal broiler chicks

Mohanna Badri[1], Samad Alimohammadi[1*], Morteza Zendehdel[2] and Shahin Hassanpour[3]

Abstract

Background Numerous physiological properties have been documented in the literature pertaining to spexin, such as its role in appetite regulation. Therefore, the present research aimed to assess the impacts of centrally administering spexin and its interaction with opioid and melanocortin receptors on feeding behavior in neonatal broiler chicks. In experiment 1, the chickens were administered intracerebroventricular (ICV) injection of saline and spexin at varying doses of 5, 7.5 and 10 nmol. During the experiment 2, the broilers were subjected to an ICV injection containing saline, β-FNA (μ-opioid receptor antagonist, 5 μg), spexin (10 nmol), and a mixture of β-FNA plus spexin. Experiments 3 to 6 bore resemblance to experiment 2, with the exception that NTI (δ-opioid receptor antagonist, 5 μg), nor-BNI (κ-opioid receptor antagonist, 5 μg), SHU9119 (MC3/MC4 receptor antagonist, 0.5 nmol) and MCL0020 (MC4 receptor antagonist, 0.5 nmol) were administered instead of β-FNA. Then, the birds were promptly placed back into their individual cages and cumulative food intake was assessed at intervals of 30, 60, and 120 min postinjection.

Results Spexin demonstrated a significant and dose-dependent decrease in food intake when compared to the control group ($P < 0.05$). Co-injection of β-FNA + spexin diminished spexin-induced hypophagia ($P < 0.05$) whereas concurrent administration of NTI and nor-BNI with spexin led to an amplification of the decrease in food consumption induced by spexin ($P < 0.05$). Additionally, anorexigenic impact of spexin was reversed by combined injection of SHU9119 and MCL0020 with spexin ($P < 0.05$).

Conclusion These observations suggest that both opioid and melanocortin receptors play crucial role in spexin-induced hypophagia.

Keywords Spexin, Opioid, Melanocortin, Food intake, Chicks

*Correspondence:
Samad Alimohammadi
S.alimohammadi@razi.ac.ir
[1]Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
[2]Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3]Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

Background

The regulation of food intake in avian species is vital for their proper growth and development and involves a complicated physiological mechanism that relies on the integration of both internal and external signals processed by hypothalamic neural networks in the central nervous system (CNS). Therefore, the study on neurotransmitters, peptides and regulatory mechanisms controlling appetite in chickens has garnered significant attention from researchers over the past few decades [1, 2].

Spexin, also known as neuropeptide Q or NPQ, is a novel neuropeptide which consists of 14 amino acids and is encoded by the C12ORF39 gene [3, 4]. Spexin was initially discovered in the human genome using a bioinformatic approach that relied on a hidden Markov model screening [5]. The evolutionary relationship among bioactive peptides spexin, galanin, and kisspeptin was validated through gene structure analysis [6]. Expression of the spexin gene and protein has been found widely in various CNS areas (such as cerebral cortex, hypothalamus, and hippocampus) as well as in peripheral tissues in human, rat, mouse and goldfish [7–10]. Meng and colleagues have recently demonstrated that spexin exhibits extensive expression throughout various chicken tissues, particularly in the CNS [11]. The various biological processes carried out by spexin are mediated via its interaction with different subtypes of galanin receptor (GALR1, GALR2 and GALR3) which belong to the family of G protein-coupled receptors (GPCRs) [12, 13]. Spexin has been proven to be implicated in mood and behavioural disorders such as anxiety and depression [14, 15], pain management [16], cardiovascular disease and endothelial function [17], renal modulation [18], regulation of reproductive axis [19], endocrine regulation [20] and obesity and diabetes [21]. Furthermore, a variety of studies have indicated the significant impact of spexin in relation to food intake and appetite regulation. For instance, in the case of meat-type chickens, when spexin is injected intracerebroventricular (ICV), it leads to a reduction in food intake by acting through galanin receptor subtypes (GALR2 and GALR3) [22]. Likewise, feeding response was noticeably inhibited after peripheral injection with spexin in chicks (*Gallus gallus*) [23]. Moreover, anorectic effect of spexin was observed in mice following its peripheral injection [24]. In this regard, administration of spexin resulted in a decrease in food consumption and subsequently facilitated weight reduction in rodents (mice and rats) with diet-induced obesity [25]. Additionally, ICV injection of spexin in goldfish led to the suppression of both feeding activity and food consumption [10].

Opioid peptides act as inhibitory neurotransmitters and are extensively distributed throughout functional neural pathways in the CNS. These peptides interact with 3 distinct subtypes of receptors known as μ-opioid receptor (MOR), δ-opioid receptor (DOR), and κ-opioid receptor (KOR), which are linked to G protein-coupled receptors (GPCRs) [26]. The opioidergic system is considered a crucial component in the regulation of food intake in birds, and it is intricately connected to other systems that play a role in controlling appetite [27]. For example, ICV injection of DAMGO (selective μ-opioid receptor agonist) was found to reduce food intake while the use of DPDPE (selective δ-opioid receptor agonist) and U-50,488 H (selective κ-opioid receptor agonist) were observed to stimulate food consumption in neonatal layer-type and meat-type chicks [28, 29]. The melanocortin peptides such as adrenocorticotropic hormone (ACTH) and various types of melanocyte-stimulating hormones (α-MSH, β-MSH, γ-MSH and δ-MSH) are derived from the precursor molecule known as proopiomelanocortin (POMC) [30]. The primary locations within the CNS where POMC is expressed predominantly are the arcuate nucleus of the hypothalamus (ARC), the anterior pituitary, and the nucleus tractus solitarius (NTS) in the brainstem [31, 32]. The melanocortin system comprises five receptor subtypes (MC1R-MC5R), classified as GPCRs, and has been demonstrated to participate in various biological pathways [33]. Several pieces of evidence suggest that the central melanocortin system plays a crucial role in regulating food intake and energy expenditure in avian species and rodents especially via MC3R/MC4R. For example, there are reports indicating that the ICV infusion of α-MSH and β-MSH resulted in an anorexigenic response in neonatal chicks [34, 35]. Furthermore, the ability of melanotan-II (MTII), a nonselective MC3R/MC4R agonist, to reduce food intake in mice following ICV administration has been documented [36].

It seems evident that there exists a functional interplay among spexin, opioid, and melanocortin receptors, which implies a complex network of communication and regulation within physiological systems. In this regard, it has been shown that spexin-induced analgesia can be reversed by naloxone and nor-BNI in formalin model of nociception in mice. Moreover, spexin through GALR3 (but not GALR2) demonstrated an up-regulation of gene and protein expression of dynorphin and κ-opioid receptor [37]. In addition, the hypophagic effect of central administration of spexin in goldfish was triggered by up-regulation of anorexigenic neuropeptides such as POMC and cocaine- and amphetamine-regulated transcript (CART) [10]. Moreover, the suppressive impact of spexin on food consumption was observed alongside a rise in the levels of MC4R expression in the hypothalamus of mice [38].

Based on findings in the previous literature and considering that spexin, opioidergic and melanocortinergic

pathways and their interaction have regulatory role in food intake and other physiological processes in animal models, the current research was conducted to explore the interaction of centrally administered spexin with opioidergic and melanocortinergic pathways on food intake regulation in neonatal broiler chicks.

Materials and methods

Animals

In the current investigation, 264 Ross-308 broiler one-day-old male chicks were included in the research sample. Experimental chicks were acquired from a local hatchery known as Morghak Company, located in Tehran, Iran. Firstly, the chickens were housed in flocks for a period of 2 days, following which they were randomly moved to separate cages and maintained based on the specified parameters of controlled temperature (30 ± 1 °C), relative humidity (50 ± 2%) and 23:1 lighting/dark schedule. Throughout the research, birds were supplied with ad libitum access to fresh water and a commercial starter diet comprising 21% crude protein and 2850 kcal/kg metabolizeable energy (Chineh Company, Tehran, Iran, Table 1). 3 h before ICV injections were administered, the chickens underwent a period of food deprivation (FD3), although they were still permitted to have unrestricted access to water. The commencement of the experiments occurred once the chickens had reached the specific age of 5 days [1, 29]. Approval for all experimental procedures and animal handling in this study were granted by the Animal Ethics Committee of Razi University, with the approval number IR.RAZI.REC.1402.027. Additionally, the aforementioned protocols were executed in compliance with the Guidelines for the Care and Utilization of Laboratory Animals in Scientific Studies [39].

Table 1 Ingredient and nutrient analysis of experimental diet

Ingredient (%)		Nutrient analysis	
Corn	52.85	ME (kcal/kg)	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 D3	0.1	Tryptophan (%)	0.20
Multi enzyme	0.05	Valine (%)	0.78

Experimental drugs

Spexin, β-funaltrexamine (β-FNA) (μ-opioid receptor antagonist), Naltrindole (NTI) (δ-opioid receptor antagonist), nor-binaltorphimine (nor-BNI) (κ-opioid receptor antagonist), SHU9119 (nonselective MC3/MC4 receptor antagonist), MCL0020 (selective MC4 receptor antagonist), and Evans Blue, along with all the other substances, were acquired from Sigma Co. (Sigma, Saint Louis, MO). In order to ready the drugs for administration, they were dissolved in a solution of 0.1% Evans Blue, which was itself formulated in a 0.85% saline solution at a proportion of 1/250. In the control group, a solution of saline mixed with Evans Blue dye was employed.

Intracerebroventricular (ICV) injection procedures

Before each treatment, the weight of the chicks was measured, and subsequently, they were classified into different experimental groups based on their body weight (BW). The allocation was performed to ensure that the average weight within the treatment groups was evenly distributed. The administration of ICV injections was conducted by employing a microsyringe (Hamilton, Switzerland) without anesthesia in accordance with the methods outlined in prior research [40, 41]. In this procedure, an acrylic apparatus was used to secure the chick's head, with the bill holder set at a 45° angle and the calvarium aligned parallel to the table surface, following the previous guideline [42]. An orifice was created in a plate positioned directly above the right lateral ventricle of the skull. The microsyringe was inserted into the ventricle through this orifice, with the needle's tip penetrating 4 mm beneath the surface of the skull and the test solution was administered [43]. Saline solution or a drug solution was injected into each chick in a volume of 10 μL [44]. Neonatal chicks do not experience any physiological stress from this injection technique [45]. At the end of the experiments, in order to assess the precision of the ICV injection, the chicks were humanely euthanized via an intraperitoneal administration of sodium thiopental overdose (50 mg/kg) (Rotexmedica, Germany; according to AVMA Guidelines for the Euthanasia of Animals 'No: S5.2.1.1', Acceptable Methods; non-inhaled agents) [46, 47]. Subsequently, the brains were excised. The confirmation of the direct injection into the lateral ventricle was fully validated by the detection of Evans Blue dye upon examination of the sliced brain tissue. It is crucial to note that data analysis only included information from individual chicks in cases where the lateral ventricle exhibited the presence of Evans Blue color. All experimental protocols were carried out between 8:00 am and 3:30 pm [1].

Feeding experiments

In this study, a total of 6 experiments were conducted on each of the 4 treatment groups. Each group comprised of at least 11 neonatal chicks, leading to a combined total of 44 chicks for every trial. The procedures outlining the treatments utilized in the experiments are detailed in Table 2. Experiment 1 was performed to evaluate the impact of ICV injection of spexin at doses of 5, 7.5 and 10 nmol on the food intake of FD3 chickens. In experiment 2, FD3 chicks ICV injected with saline, β-FNA (5 µg), spexin (10 nmol), and a combination of β-FNA and spexin. In experiment 3, ICV injections to FD3 chicks were saline, NTI (5 µg), spexin (10 nmol), and the co-administration of NTI and spexin. In experiment 4, ICV injections of the saline, nor-BNI (5 µg), spexin (10 nmol) and nor-BNI plus spexin were done in the chicks. In experiment 5, the chicks were given ICV the saline, SHU9119 (0.5 nmol), spexin (10 nmol) and a combination of SHU9119 and spexin. Lastly, in experiment 6, the chicks were ICV injected with saline, MCL0020 (0.5 nmol), spexin (10 nmol), and a combination of MCL0020 and spexin. After receiving the ICV injections, the chicks were promptly placed back into their individual cages. Fresh water and food were supplied, and the cumulative food intake (measured in grams) was recorded at 30, 60, and 120 min postinjection. The food intake was normalized by expressing it as percentage of body weight in order to reduce the impact of differences in weight among chicks on their food intake. Every chick was utilized only once within each experimental group. The doses of the substances administered via ICV injection were determined based on previous reports [22, 48, 49] and pilot studies that have not yet been published. It is noteworthy to mention that all doses that were used for experiments in this study had no behavioral changings such as sedation or hyperactivity by drugs in chicks.

Table 2 Treatments procedure in experiments 1–6

Experiment 1	**ICV Injection**
Treatment Groups	
A	Control Solution
B	Spexin (5 nmol)
C	Spexin (7.5 nmol)
D	Spexin (10 nmol)
Experiment 2	**ICV Injection**
Treatment Groups	
A	Control Solution
B	β-FNA (µ-opioid receptor antagonist, 5 µg)
C	Spexin (10 nmol)
D	β-FNA + Spexin
Experiment 3	**ICV Injection**
Treatment Groups	
A	Control Solution
B	NTI (δ-opioid receptor antagonist, 5 µg)
C	Spexin (10 nmol)
D	NTI + Spexin
Experiment 4	**ICV Injection**
Treatment Groups	
A	Control Solution
B	nor-BNI (κ-opioid receptor antagonist, 5 µg)
C	Spexin (10 nmol)
D	nor-BNI + Spexin
Experiment 5	**ICV Injection**
Treatment Groups	
A	Control Solution
B	SHU9119 (nonselective MC3/MC4
C	receptor antagonist, 0.5 nmol) Spexin (10 nmol)
D	SHU9119 + Spexin
Experiment 6	**ICV Injection**
Treatment Groups	
A	Control Solution
B	MCL0020 (selective MC4 receptor antagonist, 0.5 nmol)
C	Spexin (10 nmol)
D	MCL0020 + Spexin

Treatment Groups: $n = 11$ chicks/group

Control Solution: normal saline + 0.1% Evans Blue

ICV: Intracerebroventricular

Statistical analysis

Data were presented as mean ± SEM and subjected to analysis through a 2-way analysis of variance (ANOVA) with repeated measures. This analysis was conducted using the SPSS software version 21 for Windows (SPSS, Inc., Chicago, IL). For treatments that exhibited a notable primary impact in the ANOVA analysis, comparisons between their means were conducted utilizing the Tukey-Kramer test. The presence of significant differences between the various treatments tested in the experimental design was demonstrated using a significance level of less than 0.05 ($P < 0.05$).

Results

Figure 1 shows a simplified schematic image of the participation of central opioid and melanocortin receptors in spexin-mediated hypophagia in neonatal broiler chicks. Figure 2 demonstrates the brains of the chicks upon the conclusion of the experimental procedures to evaluate the accuracy of the ICV administration. In experiment 1, a significant and dose dependent hypophagia was noted following the ICV injection of the different doses of the spexin (5, 7.5 and 10 nmol) in comparison to the control group over the entire 120-min observation period ($P < 0.05$) (Fig. 3). In experiment 2, ICV administration of β-FNA (5 µg) had no significant effect on food intake compared to the control group ($P > 0.05$) while food intake was markedly reduced by spexin (10 nmol)

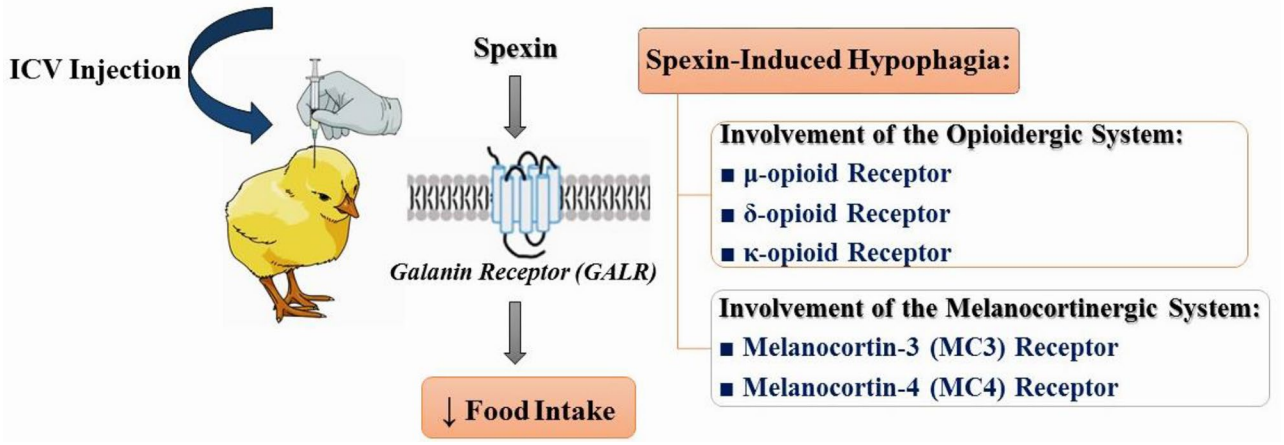

Fig. 1 Schematic image showing involvement of central opioid and melanocortin receptors in spexin-induced hypophagia in neonatal broiler chicks

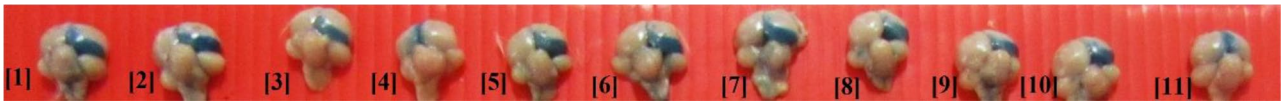

Fig. 2 Chickens' brains at the end of the experiments to assess the accuracy of the ICV injection

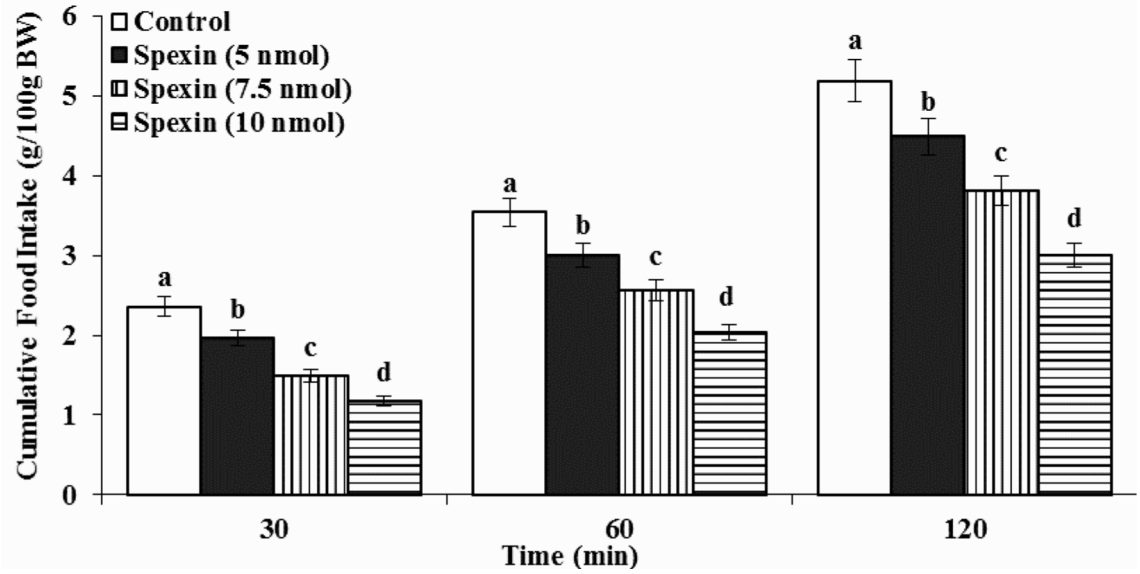

Fig. 3 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue) and different levels of the spexin (5, 7.5 and 10 nmol) on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a-d) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: $F(3,40) = 1723.13$, $P < 0.05$; time effect: $F(2,80) = 4281.06$, $P < 0.05$; treatment × time interaction: $F(6,80) = 38.53$; $P < 0.05$]

at 30, 60, and 120 min postinjection when compared to the control group ($P < 0.05$). Considerable hypophagic effect of spexin was significantly attenuated by coinjection of β-FNA + spexin ($P < 0.05$) (Fig. 4). In experiment 3, ICV injection of NTI (5 μg) did not result in any notable impact on food consumption compared to the control group ($P > 0.05$) while 10 nmol of the spexin caused a significant reduction in food intake at 30, 60, and 120 min postinjection compared to the control group ($P < 0.05$). However, coinjection of the NTI + spexin amplified spexin-induced hypophagia ($P < 0.05$) (Fig. 5). In experiment 4, ICV administration of nor-BNI (5 μg) had no significant effect on food intake compared to the control group ($P > 0.05$). Spexin (10 nmol) was associated with decreased food intake at 30, 60, and 120 min following injection in comparison to the control group ($P < 0.05$). Nevertheless, the concurrent administration of nor-BNI + spexin enhanced the hypophagic effect

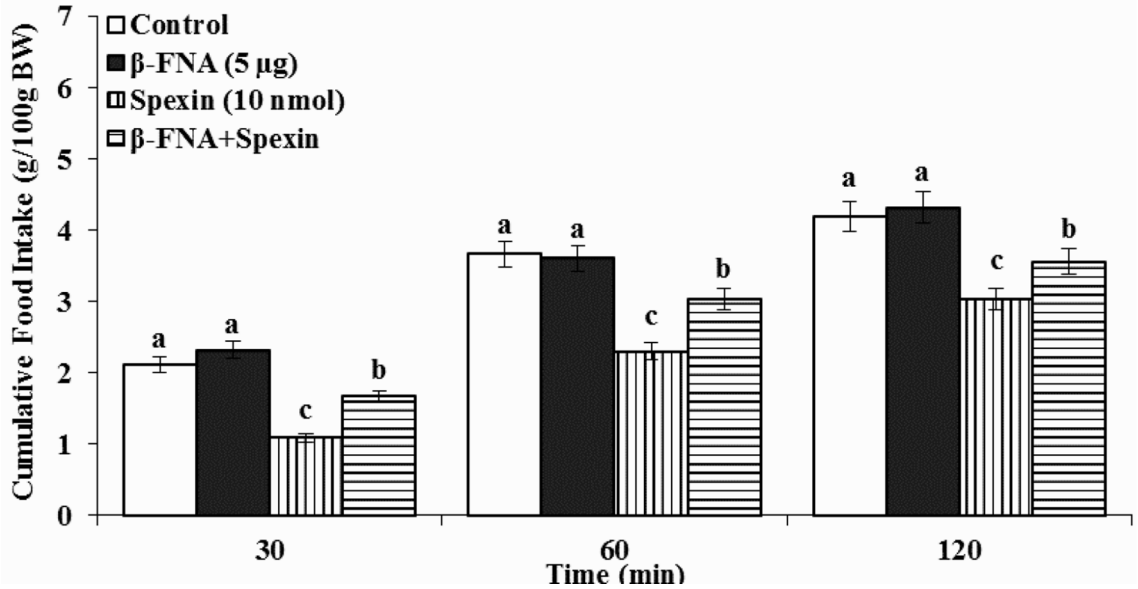

Fig. 4 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue), β-FNA (β-Funaltrexamine; μ-opioid receptor antagonist, 5 μg), spexin (10 nmol) and a combination of β-FNA plus spexin on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: F(3,40) = 2521.36, $P < 0.05$; time effect: F(2,80) = 3481.58, $P < 0.05$; treatment × time interaction: F(6,80) = 29.81; $P < 0.05$]

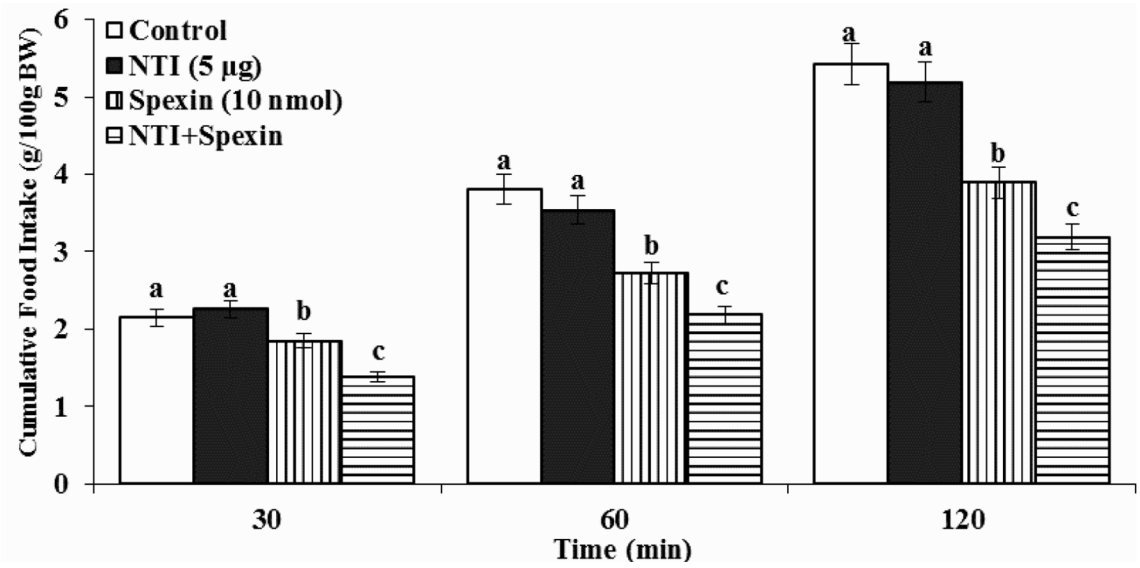

Fig. 5 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue), NTI (Naltrindole; δ-opioid receptor antagonist, 5 μg), spexin (10 nmol) and a combination of NTI plus spexin on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: F(3,40) = 3281.52, $P < 0.05$; time effect: F(2,80) = 4128.16, $P < 0.05$; treatment × time interaction: F(6,80) = 28.37; $P < 0.05$]

caused by spexin ($P < 0.05$) (Fig. 6). In experiment 5, ICV injection of SHU9119 (0.5 nmol) alone did not elicit any significant change in the food consumption compared to the control group ($P > 0.05$). The administration of spexin (10 nmol) effectively suppressed food intake up to 120 min compared to the control group ($P < 0.05$). Coinjection of the SHU9119 + spexin weakened hypophagia induced by spexin ($P < 0.05$) (Fig. 7). In experiment 6, injection of MCL0020 (0.5 nmol) via the ICV route did not result in any notable alteration in food intake when compared to the control group ($P > 0.05$). The inhibitory action of spexin (10 nmol) on food intake was observed after ICV injection up to 120 min when compared with control group ($P < 0.05$). The hypophagic effect of spexin was remarkably reversed by concurrent injection of MCL0020 + spexin ($P < 0.05$) (Fig. 8).

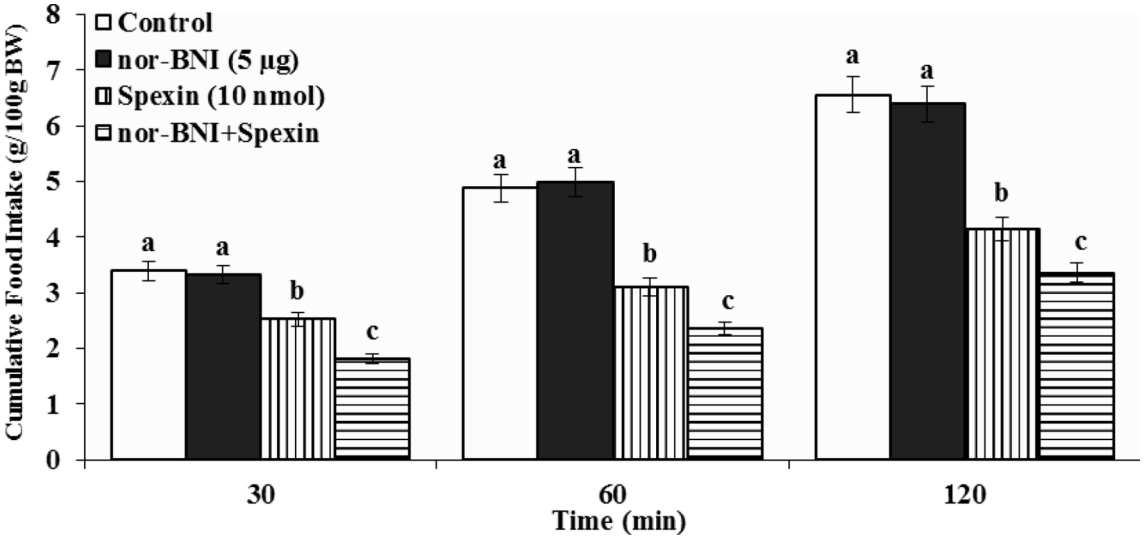

Fig. 6 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue), nor-BNI (Norbinaltorphimine; κ-opioid receptor antagonist, 5 µg), spexin (10 nmol) and a combination of nor-BNI plus spexin on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: $F(3,40) = 2894.27$, $P < 0.05$; time effect: $F(2,80) = 3841.73$, $P < 0.05$; treatment × time interaction: $F(6,80) = 26.48$; $P < 0.05$]

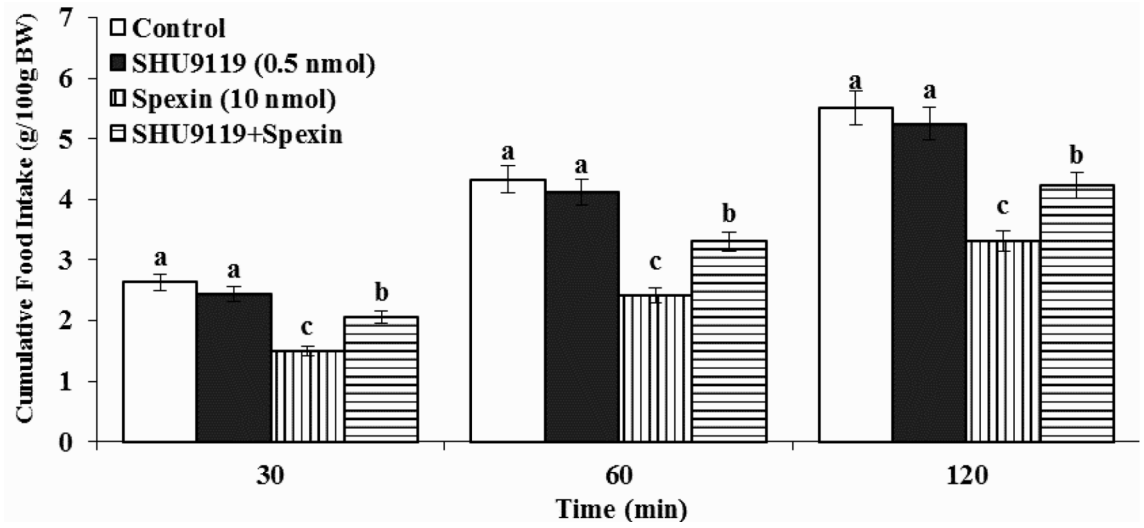

Fig. 7 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue), SHU9119 (nonselective MC3/MC4 receptor antagonist, 0.5 nmol), spexin (10 nmol) and a combination of SHU9119 plus spexin on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: $F(3,40) = 3641.92$, $P < 0.05$; time effect: $F(2,80) = 2951.45$, $P < 0.05$; treatment × time interaction: $F(6,80) = 28.63$; $P < 0.05$]

Discussion

Understanding the regulatory mechanisms of food intake, energy expenditure, and body weight in broiler birds is crucial for the development and improvement of management practices [50]. To the best of the authors' knowledge, this research provides the first report on the participation of central opioid and melanocortin receptors in spexin-induced hypophagia following ICV injection in neonatal broiler chicks. The findings from the current study in Fig. 3 clearly illustrated that the ICV injection of the spexin caused a considerable decline in cumulative food intake in a dose-dependent fashion in FD3 neonatal broiler chicks, indicating that spexin might act as a potential anorexigenic factor in the brain of broiler chicks, which was in line with prior studies that have been carried out on chickens [22, 23], rodents [24, 25], goldfish [10], zebrafish [51] and Siberian sturgeon [52]. Regarding the suppressive impact of spexin on food intake, it is possible to identify the following mechanisms. The anorexigenic effect of spexin is primarily associated with the activation of GALR2, GALR2L, and GALR3 (members of the GPCR family) within the brain, leading

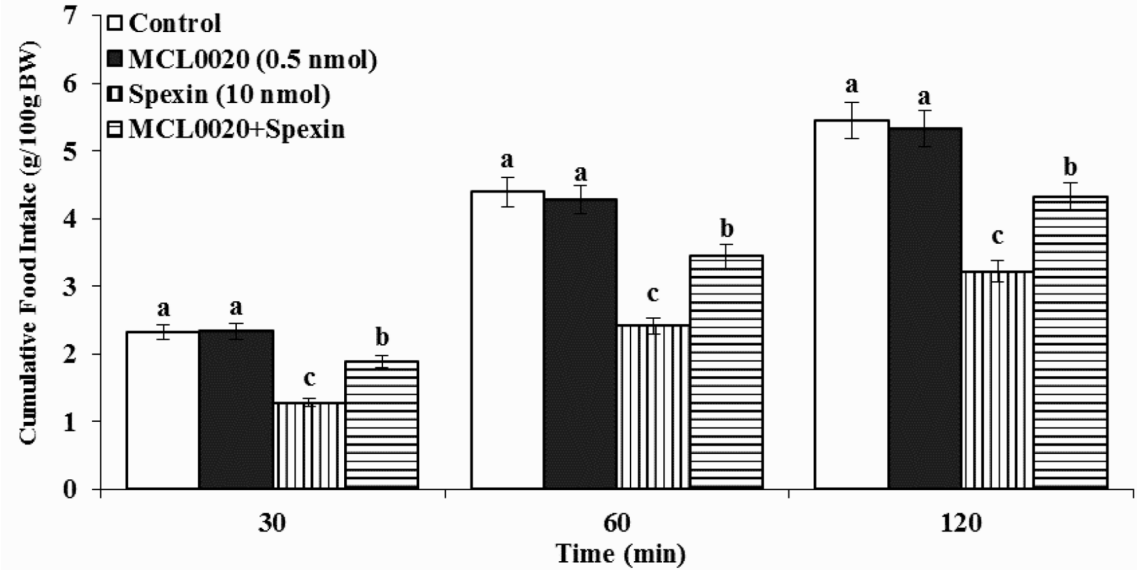

Fig. 8 Effect of ICV injection of control solution (normal saline + 0.1% Evans Blue), MCL0020 (selective MC4 receptor antagonist, 0.5 nmol), spexin (10 nmol) and a combination of MCL0020 plus spexin on cumulative food intake (g/100 g BW) in neonatal broiler chicks ($n = 11$ per group). Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$). [Treatment effect: $F(3,40) = 2847.59$, $P < 0.05$; time effect: $F(2,80) = 3475.38$, $P < 0.05$; treatment × time interaction: $F(6,80) = 24.73$; $P < 0.05$]

to the subsequent initiation of intracellular MAPK/ERK signaling cascades. Then, spexin functions as a satiety agent by reducing the levels of agouti gene-related protein (AgRP) and melanin-concentrating hormone (MCH) expressions, while simultaneously increasing the mRNA levels of cocaine- and amphetamine-regulated transcript I (CART1) in the hypothalamus [11]. Moreover, through its interaction with GALRs, spexin has the potential to induce c-Fos expression in the anterior hypothalamic area (AHA) and suprachiasmatic nucleus (SCN) via phosphorylated calmodulin kinase 2 (p-CaMK2), leading to the down-regulation of hypothalamic NPY mRNA expression, thereby resulting in the anorectic impact [24]. It is also important to mention that spexin via ICV route has the ability to decrease food intake by diminishing food-seeking behaviors, decreasing feeding traits, and enhancing food rejection activity [10, 22].

As indicated in the present study, the concurrent injection of β-FNA (β-Funaltrexamine; μ-opioid receptor antagonist) and spexin markedly reduced the hypophagic effect of spexin whereas the inhibition of food intake by spexin was amplified by coinjection of spexin with NTI (Naltrindole; δ-opioid receptor antagonist) and nor-BNI (Norbinaltorphimine; κ-opioid receptor antagonist) (Figs. 4, 5 and 6). These findings indicate the presence of a possible correlation between spexin and the opioidergic system in regulating feeding behavior in neonatal broiler chicks. The significant role of different classes of opioid receptors relevant to control of food intake has been elucidated. For example, ICV injection of DAMGO (selective μ-opioid receptor agonist) decreased cumulative food intake while DPDPE (selective δ-opioid receptor agonist) and U-50,488 H (selective κ-opioid receptor agonist) induced hyperphagia in neonatal broiler chicks [28] and neonatal layer-type chicks [29, 53]. There are functional interactions between the opioidergic system with neurotransmitters and neuropeptides in order to control feeding behavior mechanisms. For instance, it has been documented that ICV injection of β-FNA completely blocked the hypophagic effect of DAMGO in neonatal broiler chicks [54]. The hypophagic effect of serotonin was reversed by the ICV injection of the β-FNA but not by NTI and nor-BNI in neonatal broilers [55]. Furthermore, the hypophagia caused by the ICV administration of oxytocin in neonatal layer-type chicks was entirely prevented by β-FNA, whereas nor-BNI potentiated anorexigenic effect of oxytocin [48]. On the other hand, a correlation was identified between spexin and opioidergic system where ICV injection of naloxone and nor-BNI reversed the analgesic response of spexin in the mouse formalin test [37]. The connection between the opioid receptors and various other elements has been confirmed; however, the specific relationship between spexin and the opioidergic system in terms of spexin's role in reducing food intake has not been documented thus far.

Another finding uncovered during our investigation demonstrated that the coinjection of SHU9119 (MC3/MC4 receptor antagonist) and spexin resulted in the mitigation of spexin-induced hypophagia in neonatal broiler chicks (Fig. 7). Moreover, the anorexigenic impact of spexin was reduced when MCL0020 (MC4 receptor antagonist) was administered concurrently with spexin

(Fig. 8). The melanocortinergic system is recognized as another regulatory pathway that plays a role in feeding behavior and body weight in poultry, mammalian, and rodents. Nevertheless, the MC3/MC4 receptor subtypes play a crucial role in the alterations caused by melanocortin signaling [56]. In this regard, prior research has indicated that ICV administration of both agonists (MTII) and antagonists (SHU9119 and HS014 at higher doses) targeting the MC3/MC4 receptor can lead to reduced and elevated food consumption in birds, respectively [57, 58]. Furthermore, suppressive impact on food intake has been observed after ICV infusion of MC3/MC4 receptor agonists in animal models, including rodents and nonhuman primates [59]. There is a possibility of neuroendocrine interrelationships between the central melanocortin system and the hormones involved in regulating food intake and energy utilization. For example, leptin functions as a peptide with anorexigenic properties, leading to a decrease in food intake at the level of the arcuate nucleus of the hypothalamus (ARC) through the activation of melanocortin receptors [60]. Neurons in the ARC that express POMC serve as crucial components of the melanocortin system. These neurons exhibit sensitivity to circulating molecules and integrate numerous excitatory and inhibitory signals originating from diverse brain regions. Subsequently, they regulate multiple facets of feeding behavior [61]. According to functional electrophysiological investigations, kisspeptin, another member of the spexin/galanin/kisspeptin family, has been documented to excite hypothalamic anorexigenic POMC neurons through direct means involving the augmentation of Na^+/Ca^{2+} exchange and activation of nonselective cation channels [62]. Additionally, another functional link between anorexigenic neuropeptide spexin and POMC/CART neurons has been established in the brain of goldfish [10]. Likewise, the inhibitory effect of ICV injection of spexin on the food intake was noted to coincide with an increase in the levels of MC4R expression in the hypothalamus of mice [38]. Since there is a lack of available data on chicks, our ability to analyze our results in relation to previous research findings is limited.

Conclusion

In summary, these observations suggest that both opioid (via μ-, δ-, κ) and melanocortin (via MC3-, MC4) receptors play crucial role in spexin-induced hypophagia in neonatal broiler chicks. Nonetheless, it is crucial that these novel findings be utilized as the basis for upcoming research endeavors, as additional inquiries are required to elucidate the fundamental cellular and molecular signaling mechanisms that link spexin to the regulation of food intake in poultry through the opioidergic and melanocortinergic systems.

Acknowledgements
This research was supported by a grant from the Research Council of the Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. The authors would like to thank the central laboratory of the Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.

Author contributions
M.B. and S.H. operated the experimental procedure and animal handling. S.A. and M.Z. participated in acquisition and analysis of behavioral studies data, statistical analysis, project supervision and preparation of the manuscript. The final manuscript was read and approved by all authors.

Funding
This research was supported by a grant from the Research Council of the Faculty of Veterinary Medicine, Razi University, Iran.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Animal experiments in this study were approved by the Animal Ethics Committee of Razi University and followed with the Guidelines for the Care and Use of Laboratory Animals in Research (Animal Ethical Approval Number: IR.RAZI.REC.1402.027). Informed consent was obtained from the Faculty of Veterinary Medicine, Razi University and Central Laboratory of the Faculty of Veterinary Medicine, Tehran University to use the chicks in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 4 October 2024 / Accepted: 4 February 2025
Published online: 26 February 2025

References

1. Soleymani Zahed M, Alimohammadi S, Hassanpour S. Effect of intracerebroventricular (ICV) injection of adrenomedullin and its interaction with NPY and CCK pathways on food intake regulation in neonatal layer-type chicks. Poult Sci. 2024;103(7):103819.
2. Volyanskaya AR, Akberdin IR, Kulyashov MA, Yevshin IS, Romanov MN, Shagimardanova EI, et al. Kolpakov. A bird's-eye overview of molecular mechanisms regulating feed intake in chickens-with mammalian comparisons. Anim Nutr. 2024;17:61–74.
3. Wan B, Wang XR, Zhou YB, Zhang X, Huo K, Han ZG. C12ORF39, a novel secreted protein with a typical amidation processing signal. Biosci Rep. 2010;30:1–10.
4. Lv SY, Zhou YC, Zhang XM, Chen WD, Wang YD. Emerging roles of NPQ/spexin in physiology and pathology. Front Pharmacol. 2019;10:457.
5. Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, et al. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res. 2007;17:320–7.
6. Mills EG, Izzi-Engbeaya C, Abbara A, Comninos AN, Dhillo WS. Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour. Nat Rev Endocrinol. 2021;17:97–113.
7. Pałasz A, Suszka-Świtek A, Kaśkosz A, Plewka D, Bogus K, Filipczyk Ł, et al. Spexin-expressing neurons in the magnocellular nuclei of the human hypothalamus. J Chem Neuroanat. 2021;111:101883.
8. Porzionato A, Rucinski M, Macchi V, Stecco C, Malendowicz LK, De Caro R. Spexin expression in normal rat tissues. J Histochem Cytochem. 2010;58:825–37.
9. Tran A, Loganathan N, McIlwraith EK, Belsham DD. Palmitate and nitric oxide regulate the expression of spexin and galanin receptors 2 and 3 in hypothalamic neurons. Neuroscience. 2020;447:41–52.

10. Wong MK, Sze KH, Chen T, Cho CK, Law HC, Chu IK, et al. Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. Am J Physiol Endocrinol Metab. 2013;305:E348–66.
11. Meng F, Yu Y, Li J, Han X, Du X, Cao X, et al. Characterization of spexin (SPX) in chickens: molecular cloning, functional analysis, tissue expression and its involvement in appetite regulation. Poult Sci. 2023;102(1):102279.
12. Barreda-Gómez G, Giralt MT, Rodríguez-Puertas R. G protein-coupled galanin receptor distribution in the rat central nervous system. Neuropeptides. 2005;39(3):153–6.
13. Kim DK, Yun S, Son GH, Hwang JI, Park CR, Kim JI, et al. Coevolution of the spexin/galanin/kisspeptin family: spexin activates galanin receptor type II and III. Endocrinology. 2014;155:1864–73.
14. Zhuang M, Lai Q, Yang C, Ma Y, Fan B, Bian Z, et al. Spexin as an anxiety regulator in mouse hippocampus: mechanisms for transcriptional regulation of spexin gene expression by corticotropin releasing factor. Biochem Biophys Res Commun. 2020;525:326–33.
15. Pałasz A, Suszka-Świtek A, Filipczyk Ł, Bogus K, Rojczyk E, Worthington J, et al. Escitalopram affects spexin expression in the rat hypothalamus, hippocampus and striatum. Pharmacol Rep. 2016;68(6):1326–31.
16. Moazen P, Taherianfard M, Ahmadi Soleimani M, Norozpor M. Synergistic effect of spexin and progesterone on pain sensitivity attenuation in ovariectomized rats. Clin Exp Pharmacol Physiol. 2018;45(4):349–54.
17. Kumar S, Hossain MJ, Javed A, Kullo IJ, Balagopal PB. Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity. Pediatr Obes. 2018;13:374–80.
18. Toll L, Khroyan TV, Sonmez K, Ozawa A, Lindberg I, McLaughlin JP, et al. Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception. FASEB J. 2012;26:947–54.
19. Liu Y, Li S, Qi X, Zhou W, Liu X, Lin H, et al. A novel neuropeptide in suppressing luteinizing hormone release in goldfish, Carassius auratus. Mol Cell Endocrinol. 2013;374:65–72.
20. Gu L, Ma Y, Gu M, Zhang Y, Yan S, Li N, et al. Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides. 2015;71:232–9.
21. Yu M, Ju M, Fang P, Zhang Z. Emerging central and peripheral actions of spexin in feeding behavior, leptin resistance and obesity. Biochem Pharmacol. 2022;202:115121.
22. Farzin M, Hassanpour S, Zendehdel M, Vazir B, Asghari A. Effects of intracerebroventricular injection of spexin and its interaction with NPY, GalR2 and GalR3 receptors on the central food intake regulation and nutritional behavior in broiler chickens. Neurosci Lett. 2022;777:136589.
23. Meng F, Wu Y, Yu Y, Bu G, Du X, Liang Q, et al. Spexin2 is a novel food regulator in Gallus gallus. Int J Mol Sci. 2023;24(5):4821.
24. Lv S, Zhou Y, Feng Y, Zhang X, Wang X, Yang Y, et al. Peripheral spexin inhibited food intake in mice. Int J Endocrinol. 2020;2020:4913785.
25. Walewski JL, Ge F, Lobdell IVH, Levin N, Schwartz GJ, Vasselli JR, et al. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity. 2014;22:1643–52.
26. Feng Y, He X, Yang Y, Chao D, Lazarus H, Xia L. Current research on opioid receptor function. Curr Drug Targets. 2012;13(2):230–46.
27. Mahdavi K, Zendehdel M, Baghbanzadeh A. Central effects of opioidergic system on food intake in birds and mammals: a review. Vet Res Commun. 2023;47(3):1103–14.
28. Bungo T, Kawamura K, Izumi T, Dodo KI, Ueda H. Feeding responses to μ, δ and κ-opioid receptor agonists in the meat-type chick. Pharmacol Biochem Behav. 2004;78(4):707–10.
29. Alimohammadi S, Zendehdel M, Babapour V. Modulation of opioid-induced feeding behavior by endogenous nitric oxide in neonatal layer-type chicks. Vet Res Commun. 2015;39:105–13.
30. Cerdá-Reverter JM, Agulleiro MJ, Cortés R, Sánchez E, Guillot R, Leal E, et al. Involvement of melanocortin receptor accessory proteins (MRAPs) in the function of melanocortin receptors. Gen Comp Endocrinol. 2013;188:133–6.
31. Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr Rev. 1988;9:159–79.
32. Cone RD. Anatomy and regulation of the central melanocortin system. Nat Neurosci. 2005;8(5):571–8.
33. Ericson MD, Lensing CJ, Fleming KA, Schlasner KN, Doering SR, Haskell-Luevano C. Bench-top to clinical therapies: a review of melanocortin ligands from 1954 to 2016. Biochim Biophys Acta Mol Basis Dis. 2017;1863(10):2414–35.
34. Tachibana T, Sugahara K, Ohgushi A, Ando R, Kawakami SI, Yoshimatsu T, et al. Intracerebroventricular injection of agouti-related protein attenuates the anorexigenic effect of alpha-melanocyte stimulating hormone in neonatal chicks. Neurosci Lett. 2001;305:131–4.
35. Cao C, Gilbert ER, Cline MA. The anorexigenic effect of beta-melanocyte-stimulating hormone involves corticotrophin-releasing factor and mesotocin in birds. Neurosci Lett. 2020;736:135282.
36. Rowland NE, Schaub JW, Robertson KL, Andreasen A, Haskell-Luevano C. Effect of MTII on food intake and brain c-Fos in melanocortin-3, melanocortin-4, and double MC3 and MC4 receptor knockout mice. Peptides. 2010;31(12):2314–7.
37. Lv SY, Cui B, Yang Y, Du H, Zhang X, Zhou Y, et al. Spexin/NPQ induces FBJ osteosarcoma oncogene (Fos) and produces antinociceptive effect against inflammatory pain in the mouse model. Am J Pathol. 2019;189(4):886–99.
38. Wong MK, Chen Y, He M, Lin C, Bian Z, Wong AO. Mouse spexin: (II) functional role as a satiety factor inhibiting food intake by regulatory actions within the hypothalamus. Front Endocrinol. 2021;12:681647.
39. Webster J. Ethical and animal welfare considerations in relation to species selection for animal experimentation. Animals. 2014;4(4):729–41.
40. Davis JL, Masuoka DT, Gerbrandt LK, Cherkin A. Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol Behav. 1979;22:693–5.
41. Furuse M, Matsumoto M, Saito N, Sugahara K, Hasegawa S. The central corticotropin-releasing factor and glucagon-like peptide-1 in food intake of the neonatal chick. Eur J Pharmacol. 1997;339:211–3.
42. Van Tienhoven A, Juhasz LP. The chicken telencephalon, diencephalon and mesencephalon in sterotaxic coordinates. J Comp Neurol. 1962;118:185–97.
43. Jonaidi H, Noori Z. Neuropeptide Y-induced feeding is dependent on $GABA_A$ receptors in neonatal chicks. J Comp Physiol A. 2012;198:827–32.
44. Furuse M, Ando R, Bungo T, Shimojo M, Masuda Y. Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. Br Poult Sci. 1999;40:698–700.
45. Saito ES, Kaiya H, Tachibana T, Tomonaga S, Denbow DM, Kangawa K, et al. Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. Regul Pept. 2005;125:201–8.
46. Motaghi S, Jonaidi H, Talebifard H, Chavoshizadeh M. The avian central roles of alpha and beta oestrogen receptors on food intake. J Anim Physiol Anim Nutr. 2022;106(2):308–12.
47. Eslam-Aghdam T, Hassanpour S, Zendehdel M. Role of the intracerebroventricular injection α-klotho on food intake in broiler chicken: a novel study. Poult Sci. 2024;103(11):104166.
48. Raji-Dahmardeh F, Vazir B, Zendehdel M, Asghari A, Panahi N. Interaction between oxytocin and opioidergic system on food intake regulation in neonatal layer type chicken. Int J Pept Res Ther. 2019;26:1905–12.
49. Rajaei S, Zendehdel M, Rahnema M, Hassanpour S, Asle-Rousta M. Mediatory role of the central NPY, melanocortine and corticotrophin systems on phoenixin-14 induced hyperphagia in neonatal chicken. Gen Comp Endocrinol. 2022;315:113930.
50. Richards MP, Proszkowiec-Weglarz M. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poult Sci. 2007;86:1478–90.
51. Zheng B, Li S, Liu Y, Li Y, Chen H, Tang H, et al. Spexin suppress food intake in zebrafish: evidence from gene knockout study. Sci Rep. 2017;7:14643.
52. Tian Z, Xu S, Wang M, Li Y, Chen H, Tang N, et al. Identification, tissue distribution, periprandial expression, and anorexigenic effect of spexin in siberian sturgeon, Acipenser Baeri. Fish Physiol Biochem. 2020;46:2073–84.
53. Zendehdel M, Ghashghayi E, Hassanpour S, Baghbanzadeh A, Jonaidi H. Interaction between opioidergic and dopaminergic systems on food intake in neonatal layer type chicken. Int J Pept Res Ther. 2016;22:83–92.
54. Bungo T, Dodo KI, Kawamura K, Izumi T, Ueda H. Effects of various μ- and δ-opioid ligands on food intake in the meat-type chick. Physiol Behav. 2005;85(5):519–23.
55. Rahmani B, Mahdavi K, Zendedel Kheybari M, Khodadadi M, Keshavarz M, Shahabi M, et al. Role of central opioid receptors on serotonin-Induced hypophagia in the neonatal broilers. Iran J Vet Sci Technol. 2022;14(1):9–19.
56. Sharma P, Bottje W, Okimoto R. Polymorphisms in uncoupling protein, melanocortin 3 receptor, melanocortin 4 receptor, and pro-opiomelanocortin genes and association with production traits in a commercial broiler line. Poult Sci. 2008;87(10):2073–86.
57. Strader AD, Schiöth HB, Buntin JD. The role of the melanocortin system and the melanocortin-4 receptor in ring dove (Streptopelia risoria) feeding behavior. Brain Res. 2003;960(1–2):112–21.

58. Ahmadi F, Zendehdel M, Babapour V, Panahi N, Hassanpour S, Khodadadi M. Modulatory function of NMDA glutamate receptor on MC 3/MC 4 receptors agonist-induced hypophagia in neonatal meat-type chicken. Vet Res Commun. 2017;41:241–8.
59. Lu XY, Barsh GS, Akil H, Watson SJ. Interaction between α-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. J Neurosci. 2003;23(21):7863–72.
60. Cowley MA, Smart JL, Rubinstein M, Cerdán MG, Diano S, Horvath TL, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature. 2001;411(6836):480–4.
61. Nuzzaci D, Laderrière A, Lemoine A, Nédélec E, Pénicaud L, Rigault C, et al. Plasticity of the melanocortin system: determinants and possible consequences on food intake. Front Endocrinol. 2015;6:143.
62. Fu LY, van den Pol AN. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. J Neurosci. 2010;30:10205–19.

Publisher's note