The effect of the central administration of the Neuropeptide VF on feed intake and its possible interactions with glutamate and opioid systems in broiler chicken

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Abstract

Arginine-phenylalanine-amide (RFamide)-related peptides (RFRP) known as neuropeptide VF (NPVF) have a crucial role in feeding regulation. The present study purposed to determine the effect of intracerebroventricular (ICV) injection of the NPVF on dietary intake and possible interactions of NPVF with glutamate and opioid systems in broiler chicken. In experiment 1, chickens received ICV administration of the control solution, NPVF (16 nmol), 15 nmol of MK-801 (NMDA glutamate receptors antagonist), and injection of the NPVF with MK-801 together. In experiments, 2-8 chicken injected intracerebroventricularly with 390 nmol of CNQX (antagonist of AMPA glutamate receptors), 2 nmol of AIDA (an antagonist of mGLUR₁ glutamate receptors), 150 nmol of LY341495 (an antagonist of mGLUR₂ glutamate receptors), 2 nmol of UBP1112 (an antagonist for mGLUR₃ glutamate receptors), 5 µg of β-FNA (an antagonist for mu (µ) receptors), 5 µg of NTI (an antagonist for delta (δ) receptors), nor-BNI (kappa (κ) receptors antagonist; 5 µg) instead of MK-801. Following the injection, at 30, 60, and 120 min, the chick's dietary intake was recorded. As a result, NPVF (16 nmol) reduced feed intake in broilers (P<0.05). NPVF + MK-801 co-injection reduced the hypophagic action of the NPVF (P<0.05). Injection of NPVF + CNQX together reduced the anorectic action of NPVF (P<0.05). Co-injection of NPVF + β-FNA reduced effects of NPVF injection (P<0.05). Thus, in newborn broilers, NPVF-induced anorexia is probably mediated by NMDA/ AMPA glutamate and µ opioid receptors.

Introduction

Regulation of feeding behavior is a complex and precise process in the body, which requires the coordinated participation of various neurotransmitters (NTs) (Sharkey et al. 2014). (CNS)(Parker et al. 2014). Arginine-phenylalanine-amide (RFamide)-related peptides (RFRP) known as neuropeptide VF (NPVF) encoded by the NPVF gene, which corresponded to humans and animals (Moosadoost et al. 2021). At present, puberty, reproduction (Tsutsui et al. 2010), and appetite regulation (Moosadoost et al. 2021) have also been demonstrated to be affected by this neuropeptide. In this regard, in chicks, intracerebroventricular (ICV) injection of NPVF (4, 8, and 16 nmol) has hypophagic role, according to research (Cline et al. 2008; Moosadoost et al. 2021). It will also be interesting to investigate whether NPVF's connections with NTs regulating feeding affect dietary intake.

Excitatory amino acid neurotransmitter glutamate exists in the CNS, and according to our understanding, two types of its receptors (ionotropic and metabotropic receptors) have been identified. N-Methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and Kainate receptors which are ionotropic, while metabotropic receptors are mGLUR₁, mGLUR₂, as well as mGLUR₃ (Ahmadi et al. 2019). According to the previous study (Hettes et al. 2010) (Mortezaei et al. 2013), chick-eating behaviors improved and were limited after ICV delivery of NMDA receptor antagonist and AMPA receptors agonist, respectively.

Opioids are known as inhibitory neurotransmitters that have been connected to several acts, including pain adjustment, respiratory function, neuroendocrine and reward situation, and feeding regulation

(Jaefari-Anari et al. 2018). Nutrition intake was demonstrated to be changed at the time that treatments with opioid receptors agonists such as mu (μ), delta (δ), and kappa (κ) were started. According to previous investigations, ICV injections of these receptors had orectic and anorectic effects in chickens (Zendehdel et al. 2016). An interaction was reported between NPVF and the opioidergic system on pain modulating. Co-administration of the NPVF with opiates ameliorated their anti-pain effect and limited the progression of opioid-induced hyperalgesia in rats and mice (Elhabazi et al. 2017). Also, RFamide-related peptides related to gonadotropin-inhibitory hormone recreation in mammalian (Wang et al. 2018) and Glutamate fibers, placed near to axons of GnRH, have been documented to arouse and/or prohibit GnRH secretion and occlude transporter 2 of glutamate in the GnRH neurons (Angelopoulou et al. 2019; Mohapatra et al. 2021).

However, there is no information on the relation of the NPVF with glutamate and opioid systems on food intake in avians. Thus, the present study aimed to indicate the role of ICV injection of the NPVF on dietary intake and its possible interactions with glutamate and opioid systems in broiler chicken.

Materials And Methods

Animal experiment

This research included the 352 broilers that were one day of age (Ross-308) and bought from a regional incubation (Morghak Co. Iran). In a temperature of 30 plus 1°C and 50 plus 2 percent moisture, chickens were held throughout herds over two days before being randomly sent to single birdhouses (Olanrewaju et al. 2017). The animals were fed a commercialized meal comprising 21% raw protein and 2850 kcal/kg of digestible calories (Chineh Co. Iran). During the research, all birds were fed and given new water daily. Just 3 hours before the ICV administration, chickens were food-deprived (FD₃), but the water was available.

Experimental medicines

NPVF (neuropeptide VF), MK-801 (antagonist of NMDA glutamate receptors), CNQX (antagonist of AMPA glutamate receptors), AIDA (antagonist of mGLUR₁ glutamate receptors), LY341495 (antagonist of mGLUR₂ glutamate receptors), UBP1112 (antagonist of mGLUR₃ glutamate receptors), β -FNA (antagonist of μ receptors), NTI (antagonist of δ receptors), nor-BNI (antagonist of κ receptors) and Evans Blue bought from Sigma Co. (Sigma, USA). At the first step, the pharmaceuticals were solubilized in absolute dimethyl sulfoxide (DMSO) and diluted in 0.85 percent saline containing Evans blue. The ratio of DMSO was 1/250, which has no harmful impact (Qi et al. 2008).

Methods for ICV administration

Before each treatment, the birds were dedicated into trial groups based on mean body weight to equal treatment groups as much as feasible. ICV injection applied on day 5 of age utilizing a microsyringe (Hamilton, Switzerland) without anesthesia based on a method defined by Davis et al. (1979) and Furuse et al. (1997) with an acrylic device (Van Tienhoven and Juhasz, 1962). This method, ICV delivery of medicines, had no stress (Saito et al. 2005). Each neonatal chick was examined using an ICV administration (with vehicle or medicine solution) in a volume of 10 μ L pursued by slicing the frozen brain tissue for the accuracy of the injection site in the ventricle.

Nutrition trials

Eight experiments were set, with four trial groups: 1-4 (n=44 in each). In experiment 1, chickens received ICV injection of (1) control solution, (2) NPVF (16 nmol), (3) MK-801 (15 nmol), and (4) co-injection of the NPVF + MK-801. In experiment 2, birds were injected with (1) control solution, (2) NPVF (16 nmol), (3) CNQX (390 nmol), and (4) co-injection of the NPVF + CNQX. In experiment 3, chicken received: (1) control solution, (2) NPVF (16 nmol), (3) AIDA (2 nmol) and (4) co-injection of the NPVF + AIDA. In experiment four, chicks ICV injected with (1) control solution, (2) NPVF (16 nmol), (3) LY341495 (150 nmol), and (4) co-injection of the NPVF + LY341495. In experiment 5, chicks were injected with (1) control solution, (2) NPVF (16 nmol), (3) UBP1112 (2 nmol), and (4) co-injection of the NPVF + UBP1112. In experiment 6, ICV injection of the (1) control solution, (2) NPVF (16 nmol), (3) β -FNA (5 μ g), and (4) co-injection of the NPVF + β -FNA. In trial 7, birds were administrated with (1) control solution, (2) NPVF (16 nmol), (3) NTI (5 μ g), and (4) co-injection of the NPVF + NTI. In experiment 8, (1) control solution, (2) NPVF (16 nmol), (3) nor-BNI (5 μ g), and (4) co-injection of the NPVF + nor-BNI. Chicks with FD3 were instantly sent to their cages and given new water and mash meal. The total nutrition intake (in grams) was recorded at 30, 60, and 120 min following the administration. Food intake was calculated as a bodyweight percentage. In each trial group, each chick was only used once.

Statistical analysis

Accumulated dietary consumption was evaluated as a duplicated measure two-way analysis of variance (ANOVA) utilizing SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA), and the results were presented as mean $\pm$ SEM (standard error of the mean). For treatment having the primary effect by ANOVA, means compared by Tukey-Kramer test ($P < 0.05$).

Results

ICV delivery of NPVF (16 nmol) reduced feed intake after thirty, sixty, and one hundred and twenty min ($P < 0.05$). MK-801 (antagonist of NMDA glutamate receptors, 15 nmol) showed no significant impact on nutrition consumption at thirty, sixty, and one hundred and twenty min following injection ($P > 0.05$). Co-injection of NPVF + MK-801 reduced the hypophagic effect of the NPVF ($P < 0.05$) (Fig. 1).

In contrast to the controls, CNQX (antagonist of AMPA glutamate receptors, 390 nmol) had no impact on eating behavior following thirty, sixty, and one hundred and twenty min ($P > 0.05$). Central administration of NPVF (16 nmol) reduced dietary consumption after thirty, sixty, and one hundred and twenty min ($P < 0.05$). NPVF + CNQX co-injections showed an impact on hypophagia caused by NPVF after thirty, sixty, and one hundred and twenty min ($P < 0.05$) (Fig. 2).

When compared to the controls in trial III, AIDA (antagonist of mGLUR₁ glutamate receptors, 2 nmol) had no impact on eating behavior following thirty, sixty, and one hundred and twenty min ($P > 0.05$). ICV administration of NPVF (16 nmol) reduced feed consumption after thirty, sixty, and one hundred and twenty min ($P < 0.05$). After the combination of NPVF + AIDA, NPVF-induced hypophagia was not influenced after thirty, sixty, and one hundred and twenty minutes ($P > 0.05$) (Fig. 3).

ICV administration of the LY341495 (antagonist of mGLUR₂ glutamate receptors, 150 nmol) had no impact on eating behavior following thirty, sixty, and one hundred and twenty min ($P > 0.05$). ICV injection of NPVF (16 nmol) reduced feed intake after thirty, sixty, and one hundred and twenty min ($P < 0.05$). After the combination of NPVF + LY341495, hypophagia of NPVF was not influenced after thirty, sixty, and one hundred and twenty minutes ($P > 0.05$) (Fig. 4).

Thirty, sixty, and one hundred and twenty min after administration, UBP1112 (antagonist of mGLUR₃ glutamate receptors, 2 nmol) did not reduce chick-eating relative to the controls, as shown in trial 5 ($P > 0.05$). ICV delivery of NPVF (16 nmol) reduced feed intake after thirty, sixty, and one hundred and twenty min ($P < 0.05$). Thirty, sixty, and one hundred and twenty min after NPVF administration, UBP1112 showed no impact on hypophagia caused by NPVF ($P > 0.05$) (Fig. 5).

Compared to controls, β -FNA (antagonist of μ receptors, 5 μ g) injections given showed no significant impact on food consumption following thirty, sixty, and one hundred and twenty min ($P > 0.05$). ICV injection of NPVF (16 nmol) reduced chick-eating after thirty, sixty, and one hundred and twenty min ($P < 0.05$). The combination of the NPVF plus β -FNA substantially impacted NPVF -induced hypophagia ($P < 0.05$) (Fig. 6).

ICV injection of the NTI (antagonist of δ receptors, 5 μ g) had no impact on eating behavior following thirty, sixty, and one hundred and twenty min ($P > 0.05$). ICV injection of NPVF (16 nmol) reduced chicken-eating after thirty, sixty, and one hundred and twenty min ($P < 0.05$). After administration of NPVF + NTI, hypophagia of NPVF was not influenced after thirty, sixty, and one hundred and twenty minutes ($P > 0.05$) (Fig. 7).

Injection of the nor-BNI (antagonist of κ receptors, 5 μ g) had no impact on eating behavior following ($P > 0.05$). ICV injection of NPVF (16 nmol) reduced feed intake after thirty, sixty, and one hundred and twenty min ($P < 0.05$). The combination of the NPVF with nor-BNI was not influenced hypophagia by NPVF after thirty, sixty, and one hundred and twenty minutes ($P > 0.05$) (Fig. 8).

Discussion

To the best of our knowledge, this is the first work aiming at ICV injection of the NPVF on dietary intake and its possible interactions with glutamate and opioid systems in broiler chicken. The induced balance between dietary intake and energy consumption resulting in the interaction of peripheral inputs with CNS is known as energy homeostasis. NTs and their regulatory nexus have a pivotal role in energy homeostasis in this regard (Rahmani et al. 2021). Based on the results obtained, NPVF (16 nmol) can induce decreased feed intake in broilers after three hours of feeding restriction. NPVF has a high affinity to the NPFF1 receptor and affects eating behavior in animals (Tachibana et al. 2005; 2007). It is found that ICV delivery of RFRP-3 promoted rat eating (Murakami et al. 2008). RFRP-1 and RFRP-3, on the other hand, limited chick-eating (Newmyer and Cline. 2009; Cline et al. 2008). Observed differences might have related to millions of years' discrepancy on evolution between avian and mammalian (Hassanpour et al. 2015). Neurons of RFRP-3 are projected to the POMC and agouti-related peptide (AgRP) neurons which are placed in the arcuate nucleus, and it is a well-known fact that both neuron nexus have a crucial role in relish and dietary consumption control in avians (Moosadoost et al. 2021). Further, **it is suggested that anorexigenic peptides such as a melanocyte-stimulating hormone or glucagon-like peptide-1 have an important role than the hypothalamic-pituitary-adrenal axis for the temporary anorexia of RFamides** (Cline et al. 2008). For instance, Moosadoost et al. (2021) reported that ICV injection of the RFRP-3 (4, 8, and 16 nmol) significantly decreased feeding in broiler chicken, and our result agrees with this report.

As observed, co-injection of NPVF + NMDA receptors antagonist reduced the anorectic effect of the NPVF. Also, the combination of NPVF + AMPA receptors antagonist reduced the anorectic effect of NPVF while MGLUR₁ / mGLUR₂ / mGLUR₃ glutamate receptors antagonists did not affect NPVF-induced hypophagia. Further, in previous studies, ICV injection of MK-801, CNQX, and β -FNA had a regulatory impact on NTs involved in dietary intake in chicks; however, AIDA, LY341495, UBP1112 had no effect (Mobarhan et al. 2021; Adeli et al. 2020; Mortezaei et al. 2013). It appears that there is a connection between glutamate and NPVF on eating behavior resulting from an association between the NPVF and glutamate. According to this notion, the blockage of transporter 2 of glutamate in the GnRH neurons is influenced by RFamide-related peptides (Mohapatra et al. 2021).

Co-injection of NPVF plus μ -receptors antagonist reduced effects of NPVF injection. δ / κ receptors antagonist, on the other hand, did not influence NPVF -induced hypophagia. Also, in a previous investigation, ICV administration of MK-801, CNQX, and β -FNA had a regulatory impact on NTs in feeding neonatal chicks; however, AIDA, LY341495, UBP1112, and NTI had none (Raji- Dahmardeh et al. 2020). It turns out to be an interaction between opioids and NPVF on dietary intake behavior, based on a relationship between the NPVF and analgesic effects of opiates. In this view, it is reported that the function of the opioidergic system is modulated by antagonizing the neuropeptide FF (NPFF) receptors. Activation of NPFF receptors decreases the anti-pain impacts of opiates and prevents conditioned test preferences caused by morphine (Kim et al. 2016). In addition, μ -opioids suppresses vasopressin and oxytocin neurons in the hypothalamic supraoptic nucleus. NPFF receptors and μ -receptors are both expressed in the supraoptic nucleus. It is assumed that RFRP-3 notably alleviates the prohibitory impact of morphine on both neurons of vasopressin and oxytocin (Kim et al. 2016). Also, on the report of

previous discoveries, it has been indicated that opioid receptors can affect NPFF-induced rat-eating behavior (Nicklous and Simansky, 2003).

The results showed that in chicks, NPVF-induced hypophagia was mediated by NMDA/AMPA and μ -receptors. However, further investigation is remained to be determined the key cellular and molecular signaling pathways in the links between NPVF-induced hypophagia with NMDA /AMPA glutamate and μ opioid receptors in chick feeding behavior.

Declarations

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Author Contributions Behnam Hamidi, Morteza Zendehtdel, Bita Vazir, and Ahmad Asghari wrote the main manuscript text and prepared figures 1-8. All authors reviewed the manuscript. The work is original and not previously published. All data are true and accurate to the knowledge of the authors.

Data availability Data will be made available upon request.

Compliance with Ethical Standards

Conflict of interest Authors has no potential conflicts of interest.

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

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

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Figures

Figure 1

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), MK-801 (NMDA glutamate receptors antagonist; 15 nmol), and co-injection of the NPVF plus MK-801 on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 2

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), CNQX (AMPA glutamate receptors antagonist; 390 nmol), and co-injection of the NPVF plus CNQX on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 3

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), AIDA (mGLUR₁ glutamate receptors antagonist; 2 nmol), and co-injection of the NPVF plus AIDA on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ 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, NPVF (neuropeptide VF; 16 nmol), LY341495 (mGLUR₂ glutamate receptors antagonist; 150 nmol), and co-injection of the NPVF plus LY341495 on cumulative food intake (g/100g BW) in neonatal chicks. Data are expressed as mean $\pm$ SEM. Different letters (a and b) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 5

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), UBP1112 (mGLUR₃ glutamate receptors antagonist; 2 nmol), and co-injection of the NPVF plus UBP1112 on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ SEM. Different letters (a and b) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 6

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), β-FNA (mu receptors antagonist; 5 μg), and co-injection of the NPVF plus β-FNA on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 7

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), NTI (delta receptors antagonist; 5 µg), and co-injection of the NPVF plus NTI on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ SEM. Different letters (a and b) indicate significant differences between treatments at each time ($P < 0.05$)

Figure 8

Effects of intracerebroventricular injection of control solution, NPVF (neuropeptide VF; 16 nmol), nor-BNI (kappa receptors antagonist; 5 µg), and co-injection of the NPVF plus nor-BNI on cumulative food intake (g/100g BW) in neonatal meat-type chicks. Data are expressed as mean $\pm$ SEM. Different letters (a and b) indicate significant differences between treatments at each time ($P < 0.05$)