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

Behnam Hamidi1 · Morteza Zendehdel2 · Bita Vazir1 · Ahmad Asghari3

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Abstract
Arginine-phenylalanine-amide (RFamide)-related peptides 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 chickens were 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 the 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.

Keywords Neuropeptide VF · Glutamate · Opioid · Feed intake · Broiler chicks

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). In the 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 (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 mammalians (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 transport 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 (Mahan 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 h 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). In 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; Blevins et al. 2002). Evans blue containing DMSO/Saline was utilized as the control group.

**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). At the final step of the experiments, the chickens were decapitated to assess the accuracy of the injection. The injection placement accuracy in the lateral ventricle was confirmed by the Evans blue staining of the sliced frozen tissue of the brain. All chickens were injected only once and only the data obtained from chickens with Evans Blue dye in the lateral ventricle were used for analysis (each group had 11 chickens). Each experiment was performed on chickens from 08:00 a.m. to 1:30 p.m. on the designated day for that experiment.

**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 (DMSO/Saline mixture), (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 (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) CNQX (390 nmol), and (4) co-injection of the NPVF + CNQX. In experiment 3, chicken received: (1) control solution (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) AIDA (2 nmol) and (4) co-injection of the NPVF + AIDA. In experiment four, chicks ICV were injected with (1) control solution (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) LY341495 (150 nmol), and (4) co-injection of the NPVF + LY341495. In experiment 5, chicks were injected with control solution (DMSO/Saline mixture), (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 (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) β-FNA (5 µg), and (4) co-injection of the NPVF + β-FNA. In trial 7, birds were administrated
with (1) control solution (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) NTI (5 µg), and (4) co-injection of the NPVF + NTI. In experiment 8, (1) control solution (DMSO/Saline mixture), (2) NPVF (16 nmol), (3) nor-BNI (5 µg), 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. The doses of drugs have been established by pilot and previous studies (Mortezaei et al. 2013; Raji-Dahmardeh et al. 2020; Adeli et al. 2020; Moosadoost et al. 2021; Mobarhan et al. 2021).

**Statistical Analysis**

Accumulated dietary consumption was evaluated as a repeated 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 ± 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 mGLUR$_1$ 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 mGLUR$_2$ 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 (agonist of \(\mu\) receptors, 5 µg) 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 (agonist of δ receptors, 5 µg) 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 (agonist of κ receptors, 5 µg) had no impact on eating behavior following ($P > 0.05$). 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 + nor-BNI, hypophagia of NPVF was not influenced 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 (Bonini et al. 2000; Liu et al. 2001) and affects eating behavior in animals (Tachibana et al. 2005). 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 (Cline et al. 2008; Newmyer and Cline. 2009). Observed differences might have related to millions of years' discrepancy on evolution between avians and mammalians (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, according to previous studies (Anand and Brobeck 1951; Tachibana et al. 2007; Cline et al. 2008), it seems 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. 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.

It appears that there is a connection between glutamate and NPVF on feeding 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).

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 and CNQX had a
regulatory impact on NTs involved in dietary intake in chicks, which matched with this report; however, AIDA, LY341495, UBP1112 had no effect (Mortezaei et al. 2013; Adeli et al. 2020; Mobarhan et al. 2021).

Also, 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 suppress 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).

As observed, co-injection of NPVF plus μ-receptors antagonist reduced the 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 β-FNA had a regulatory impact on NTs in feeding neonatal chicks, which our outcome conforms with this report.; however, NTI and nor-BNI had no effect (Raji-Dahmardeh et al. 2020). The results showed that, NPVF-induced anorexia is probably mediated by NMDA/AMPA glutamate and μ opioid receptors in chicks. 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 feeding behavior of chickens.

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Declarations

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Conflict of interest Authors has no potential conflicts of interest.

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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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