CRF<sub>1</sub>/CRF<sub>2</sub> and MC<sub>3</sub>/MC<sub>4</sub> Receptors Affect Glutamate-Induced Food Intake in Neonatal Meat-Type Chicken

**ABSTRACT**

Central glutamate, melanocortin and corticotropin systems have mediatory role on several physiologic functions in the brain, but their interactions on appetite regulation are not fully elicited. So, the aim of the current study was to determine interaction of the glutamate with melanocortin and corticotropin systems on food intake in 3-h food-deprived (FD<sub>3</sub>) neonatal meat-type chicken. In experiment 1, chicken intracerebroventricular (ICV) injected (A) phosphate-buffered saline (PBS), (B) glutamate (75 nmol), (C) glutamate (150 nmol) and (D) glutamate (300 nmol). In experiment 2, (A) PBS, (B) astressin-B (CRF<sub>1</sub>/CRF<sub>2</sub> receptors antagonist, 30 µg), (C) glutamate (300 nmol) and (D) astressin-B+glutamate were ICV injected. Experiments 3-5 were similar to experiment 2, except birds were injected with astressin2-B (CRF<sub>1</sub> receptor antagonist, 30 µg), SHU9119 (MC<sub>3</sub>/MC<sub>4</sub> receptor antagonist, 0.5 nmol) and MCL0020 (MC<sub>4</sub> receptor antagonist, 0.5 nmol) instead of the astressin-B. In experiment 6, the injections were (A) PBS, (B) MTII (MC<sub>3</sub>/MC<sub>4</sub> receptor agonist, 2.5ng), (C) glutamate (75nmol) and (D) MTII+glutamate. Then, cumulative feed intake was recorded at 30, 60 and 120 minutes after injection. According to the results, dose dependent hypophagia observed by ICV injection of the glutamate (75, 150 and 300nmol) compared to control group in neonatal broiler chicken (p<0.05). Co-injection of the astressin-B+glutamate and astressin2-B+glutamate decreased glutamate-induced hypophagia in neonatal broiler chicken (p<0.05). Co-injection of the glutamate+MC<sub>3</sub>/MC<sub>4</sub> receptors antagonist decreased hypophagic effect of the glutamate (p<0.05). These results suggested hypophagic effect of the glutamate mediates via CRF<sub>1</sub>/CRF<sub>2</sub> and MC<sub>3</sub>/MC<sub>4</sub> receptors in chickens.

**INTRODUCTION**

Feed intake, satiety and energy expenditure regulates via diverse signals from central and peripheral tissues (Hassanpour et al., 2015; Zendehdel et al., 2017). Neurotransmitters interact by a wide distributed neurological network on feed intake regulation in the central nervous system (CNS) (D’Addario et al., 2014). Appetite regulation regulates in several brain areas such as striatum, hypothalamus, amygdala, nucleus tractus solitarius (NTS) and arcuate nucleus (ARC) (D’Addario et al., 2014).

The melanocortin system is one of the central neurotransmitter systems and to date its five subtypes (MC<sub>R</sub>-MC<sub>5</sub> R) have been identified (Alvaro et al., 2003). It has prominent role in several physiologic functions e.g. grooming, thermoregulation, learning and energy balance regulation (Schneeberger et al., 2014). In the brain of the avian Melanocortin receptors have also, been identified (Takeuchi et
Among melanocortin receptors only MC₃R and MC₄R subtypes are responsible for the central feed intake regulation (Schneeberger et al., 2014). The MC₅R and MC₆R mainly found in arcuate nucleus (ARC), ventromedial hypothalamus (VMH) and periventricular nucleus (PVN) regions of the hypothalamus (Liu et al., 2003). It is reported ICV injection of the MC₃R / MC₄R receptors agonists decreased feed intake in rats (Strader et al., 2003).

Corticotrophin-releasing factor (CRF) is a 41 amino acid peptide and has major role in regulating central aspects of the stress response (Silberman and Winder, 2013). Corticotrophin receptors (CRF₁ and CRF₂) are G-protein-coupled receptors and regulators pituitary function in anxiety and stress (Yamada and Bruijnzeel, 2011). Activation of the CRF₁ and CRF₂ receptors decreases feed intake (Richard et al. 2002). The ICV injection of the Astressin-B or Astressin2-B decreased feed intake in rats (Stengel et al., 2009). Also, Finelli et al., (2014) reported ICV injection of the Astressin2-B decreased feed intake in rats (Finelli et al., 2014). Recently, Heidarzadeh et al., (2017) reported ICV injection of the Astressin-B affects nesfatin-1 induced hypophagia in FD₃ neonatal broiler chicken.

Glutamate is the main excitatory neurotransmitter in the CNS and its two classified subtypes, the ionotropic and metabotropic receptors have been identified. N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and Kainate receptors belongs to the ionotropic while mGLUR₉, mGLUR₁, and mGLUR₃ receptors are metabotropic (Baghbanzadeh and Babapour, 2007). In several reports, effect of both ionotropic and metabotropic receptors on feeding behavior is identified (Zeni et al., 2000; Baghbanzadeh and Babapour, 2007; Zendehdel et al., 2009). Hypophagia reported by ICV injection of AMPA receptors agonist into the lateral hypothalamus in mammals (Ciranna, 2006; Hettes et al., 2010). Also, the ICV injection of the DL-AP5 (NMDA receptor antagonist) increased food consumption in FD₃ broiler cockerels (Taati et al., 2011; Mortezaei et al., 2013). Injection of the AMPA, NMDA and kainate receptors antagonist into the brain increased feeding behavior in pigeon (Da Silva et al., 2003).

Both melanocortin and glutamatergic neurons are identified in the nucleus of the tractus solitarius (NTS), PVN, amygdala, ARN and area postrema of the brain which engaged with feed intake regulation centers in mammalian (Liu et al., 2003). In rats VTA, excitatory glutamatergic transmission is potentiated through CRF₁ modulation of the NMDA transmission (Ungless et al., 2003). Also, CRF increase glutamatergic neurotransmission in the central amygdala (Silberman and Winder, 2013). According to the reports there are differences in central food intake regulation mechanisms between mammals and avian (Richards, 2003). There is no report on the interaction of glutamate with melanocortin and corticotropin systems on appetite regulation in avian. Based on comparative physiology, it is logical to assume the regulatory mechanisms governing these processes in birds (Furuse et al., 2007). Therefore, the main purpose of the current study was to determine interaction of the glutamate with melanocortin and corticotropin systems on feed intake in FD₃ neonatal meat-type chicken.

**MATERIAL AND METHOD**

**Animals**

A total of 264 one-day-old male meat-type chickens were purchased from a local hatchery (Mahan Co., Iran). Birds were maintained in stabilizing electrically heated batteries at a temperature of 32 ºC ± 1, kept at 40-50 %relative humidity and 23:1 lighting/dark period (Olanrewaju et al., 2006). They were kept for 2 days as flocks and then the birds were randomly allocated and transferred into their individual cages. A commercial starter diet containing 21% crude protein and 2850 kcal/kg metabolizable energy (Animal Science Research Institute Co. Iran) were provided to the animals (table 1). During the study all birds had ad libitum access to food and fresh water. 3 h prior to the injections, the birds were food deprived (FD₃) but had free access to water. ICV injections were done at 5 days of age. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, USA (publication No. 85-23, revised 1996) and the current laws of the Iranian government for animal care, and were approved by the Institutional Animal Ethics Committee of Faculty of Veterinary Medicine, University of Tehran.

**Experimental drugs**

Drugs used include glutamate, astressin-B (CRF₁/CRF₂ receptors antagonist), astressin2-B (CRF₂ receptor antagonist), SHU9119 (MC₃/MC₄ antagonist), MCL0020 (MC₄ receptor antagonist), MTII (MC₃/MC₄ agonist) and Evans blue. They were purchased from...
In this study, six experiments were designed to determine interaction of the glutamate with CRF1 / CRF2 and MC3 / MC4 receptors in neonatal meat-type chicken. (Each experiment included 4 groups with 11 replicates in each group; n=44). In experiment 1, chicken ICV injected with (A) phosphate-buffered saline (PBS), (B) glutamate (75 nmol), (C) glutamate (150 nmol) and (D) glutamate (300 nmol). In experiment 2, (A) PBS, (B) astressin-B (30 µg), (C) glutamate (300 nmol) and (D) astressin-B + glutamate were ICV injected. In experiment 3, birds ICV injected with (A) phosphate-buffered saline (PBS), (B) astressin2-B (30 µg), (C) glutamate (300 nmol) and (D) astressin2-B + glutamate. In experiment 4, FD3 chicks received ICV injection of (A) PBS, (B) SHU9119 (0.5 nmol), (C) glutamate (300 nmol) and (D) SHU9119 + glutamate. In experiment 5, the ICV injection to the birds were (A) PBS, (B) MTII (2.5 ng), (C) glutamate (75 nmol) and (D) MTII + glutamate. The injection procedure in the experimental procedure is presented in table 2 and in the flow chart. Immediately after the injection feed was provided to the birds and cumulative feed intake (g) was measured at 30, 60 and 120 min after the injection. Food consumption was calculated as a gram of body weight (g/100g BW) to minimize the impact of body weight on the amount of feed intake. These doses of drugs were determined according to the pilot and previous studies (Zeni et al., 2000; Baghbanzadeh & Babapour, 2007; Zendehdel et al., 2009; Ahmadi et al., 2017; Heidarzadeh et al., 2017).

**Table 1 – Ingredient and nutrient analysis of experimental diet.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(%)</th>
<th>Nutrient analysis</th>
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<tbody>
<tr>
<td>Corn</td>
<td>52.85</td>
<td>ME, kcal/g 2850</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>31.57</td>
<td>Crude protein (%) 21</td>
</tr>
<tr>
<td>Wheat</td>
<td>5</td>
<td>Linoleic acid (%) 1.69</td>
</tr>
<tr>
<td>Gluten meal, 61% CP</td>
<td>2.50</td>
<td>Crude fiber (%) 3.55</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>2.47</td>
<td>Calcium (%) 1</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.92</td>
<td>Available phosphorus (%) 0.5</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>1.23</td>
<td>Sodium (%) 0.15</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.00</td>
<td>Potassium (%) 0.96</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.25</td>
<td>Chlorine (%) 0.17</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.25</td>
<td>Choline (%) 1.30</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.21</td>
<td>Arginine (%) 1.14</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.20</td>
<td>Isoleucine (%) 0.73</td>
</tr>
<tr>
<td>Acidifier</td>
<td>0.15</td>
<td>Lysine (%) 1.21</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.10</td>
<td>Methionine (%) 0.49</td>
</tr>
<tr>
<td>Toxin binder</td>
<td>0.10</td>
<td>Methionine + cysteine (%) 0.83</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.05</td>
<td>Threonine (%) 0.70</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>0.1</td>
<td>Tryptophan (%) 0.20</td>
</tr>
<tr>
<td>Multi enzyme</td>
<td>0.05</td>
<td>Valine (%) 0.78</td>
</tr>
</tbody>
</table>

ME: metabolisable energy, CP: crude protein, per kg of diet, the mineral supplement contains 35.2 g manganese from MnSO4.H2O; 22 g iron from FeSO4.H2O; 35.2 g zinc from ZnO; 4.4 g copper from CuSO4.5H2O; 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g selenium from Na2SeO3. 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 pyridoxin, 0.022 g of biotin, 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g selenium from FeSO4∙H2O; 35.2 g zinc from ZnO; 4.4 g copper from CuSO4∙5H2O; 35.2 g manganese from MnSO4∙H2O; 22 g iron from FeSO4∙H2O. The control group received a control solution (saline containing Evans blue, 10 µL) (Furuse et al., 1999). This technique does not induce any physiological stress in neonatal chicks (Saito et al., 2005). At the end of the experiments, to recognize the accuracy of the injection, the chicks were sacrificed by decapitation. Accuracy of placement of the injection in the ventricle was verified by the presence of Evans blue followed by slicing the frozen brain tissue. In each group, 12 birds received the injection, but just the data of those individuals where dye was present in their lateral ventricle were used for analysis (11 chickens per group). All experimental procedures were done from 8:00 A.M. until 3:30 P.M.

**Feeding experiments**

In each experiment, the birds were weighed and based on their body weight allocated into experimental groups so the average weight between treatment groups was as uniform as possible. The chicken was ICV injected once in each experiment using a microsyringe (Hamilton, Switzerland) without anesthesia using the Davis et al., (1979) and Furuse et al., (1997) method. Briefly, the head of the chicken was held with an acrylic device in which the bill holder was 45º and the calvarium was parallel to the surface of the table as explained by Van Tienhoven & Juhasz (1992). An orifice was made in a plate over the skull of the right lateral ventricle. A microsyringe was inserted into the ventricle through the orifice in the plate and the tip of the needle perforated only 4 mm below the skin of the skull (Jonaidi & Noori, 2012). All injections were done in a volume of 10 µL (Furuse et al., 1999).
Statistical analysis

Cumulative feed intake was analyzed by repeated measure two-way analysis of variance (ANOVA) and is presented as the mean ± SEM. For treatments found to have an effect according to the ANOVA, mean values were compared with Bonferroni test. P values <0.05 were considered to indicate significant differences between the treatments.

RESULTS

Effects and interactions of central glutamate with CRF₁, CRF₂, MC₃ and MC₄ receptors on cumulative feed intake in FD₃ neonatal broilers are shown in figures 1-6. In experiment 1, dose dependent hypophagia was observed by ICV injection of the different doses of the glutamate (75, 150 and 300 nmol) compared to the control group in neonatal broiler chicken [treatment effect: F (3, 80) = 385.1, P<0.0001; time effect: F (2, 80) = 824.7, p<0.0001; treatment and time interaction: F (6, 80) = 73.42; p<0.0001; Fig. 1].

In experiment 2, ICV injection of the astressin-B (30 µg) had no effect on feed intake (P>0.05). The glutamate injection (300 nmol) significantly decreased feed intake compared to the control group (P<0.05). Co-injection of the astressin-B + glutamate, decreased glutamate-induced hypophagia in neonatal broiler chicken [treatment effect: F (3, 80) = 241.7, p<0.0001; time effect: F (2, 80) = 541.8, p<0.0001; treatment and time interaction: F (6, 80) = 35.18; p<0.0001; Fig. 2].

In experiment 3, ICV administration of the astressin2-B (30 µg) had no effect on feeding behavior (P>0.05) while 300 nmol of the glutamate significantly decreased feed intake compared to the control group (P<0.05). Co-injection of the astressin-B + glutamate, decreased glutamate-induced hypophagia in neonatal broiler chicken [treatment effect: F (3, 80) = 241.7, p<0.0001; time effect: F (2, 80) = 541.8, p<0.0001; treatment and time interaction: F (6, 80) = 35.18; p<0.0001; Fig. 2].

Figure 1 – Effects of intracerebroventricular injection of control solution (PBS) and different doses of the glutamate (75, 150 and 300 nmol) on cumulative food intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a-c) indicate significant differences between treatments at each time (p<0.05).

Figure 2 – Effects of intracerebroventricular injection of control solution (PBS), astressin-B (CRF₁/CRF₂ receptors antagonist; 30 µg), glutamate (300 nmol) and co-injection of the astressin-B + glutamate on cumulative feed intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time (p<0.05).

Table 2 – Treatments procedure in experiments 1-7

<table>
<thead>
<tr>
<th>Experiment</th>
<th>ICV Injection</th>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>solution (PBS) *</td>
<td>I solution (PBS) *</td>
</tr>
<tr>
<td></td>
<td>glutamate (75 nmol)</td>
<td>II glutamate (75 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (150 nmol)</td>
<td>III glutamate (150 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol)</td>
<td>IV glutamate (300 nmol)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>solution (PBS) *</td>
<td>I solution (PBS) *</td>
</tr>
<tr>
<td></td>
<td>astressin-B (30 µg)</td>
<td>II astressin-B (30 µg)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol)</td>
<td>III glutamate (300 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol) + astressin-B (30 µg)</td>
<td>IV glutamate (300 nmol) + astressin-B (30 µg)</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>solution (PBS) *</td>
<td>I solution (PBS) *</td>
</tr>
<tr>
<td></td>
<td>SHU9119 (0.5 nmol)</td>
<td>II SHU9119 (0.5 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol)</td>
<td>III glutamate (300 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol) + SHU9119 (0.5 nmol)</td>
<td>IV glutamate (300 nmol) + SHU9119 (0.5 nmol)</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>solution (PBS) *</td>
<td>I solution (PBS) *</td>
</tr>
<tr>
<td></td>
<td>MCL0020 (0.5 nmol)</td>
<td>II MCL0020 (0.5 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol)</td>
<td>III glutamate (300 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (300 nmol) + MCL0020 (0.5 nmol)</td>
<td>IV glutamate (300 nmol) + MCL0020 (0.5 nmol)</td>
</tr>
<tr>
<td>Experiment 5</td>
<td>solution (PBS) *</td>
<td>I solution (PBS) *</td>
</tr>
<tr>
<td></td>
<td>MTII (2.5 ng)</td>
<td>II MTII (2.5 ng)</td>
</tr>
<tr>
<td></td>
<td>glutamate (75 nmol)</td>
<td>III glutamate (75 nmol)</td>
</tr>
<tr>
<td></td>
<td>glutamate (75 nmol) + MTII (2.5 ng)</td>
<td>IV glutamate (75 nmol) + MTII (2.5 ng)</td>
</tr>
</tbody>
</table>

PBS: phosphate-buffered saline, astressin-B: CRF₁/CRF₂ receptors antagonist, astressin2-B: CRF₂ receptor antagonist, SHU9119: MC₃/MC₄ antagonist, MCL0020: MC₄ receptor antagonist, MTII (MC₃/MC₄ agonist).
In experiment 4, ICV administration of the 0.5 nmol MC3/MC4 antagonist (SHU9119) had no effect on feeding behaviour (P >0.05) while the glutamate injection (300 nmol) significantly decreased feed intake compared to control group (p<0.05). Co-injection of the SHU9119 + glutamate decreased the hypophagic effect of the glutamate in neonatal chicks [treatment effect: F (3, 80) = 240.27, p<0.0001; time effect: F (2, 80) = 51.17, p<0.0001; treatment and time interaction: F (6, 80) = 163.72, p<0.0001; time effect: F (2, 80) = 628.15, p<0.0001; treatment and time interaction: F (6, 80) = 439.52, p<0.0001; treatment effect: F (2, 80) = 376.08, p<0.0001; time effect: F (2, 80) = 11.06, p<0.0001; Fig. 5].

In experiment 5, MCL0020 (0.5 nmol) had no effect on feeding behavior in FD3 neonatal broilers compared to control group (P >0.05) while the glutamate injection (300 nmol) had hypophagic effect in FD2 neonatal broilers compared to the control group (p<0.05). Co-administration of the glutamate + MCL0020 significantly diminished glutamate-induced hypophagia in chicks [treatment effect: F (3, 80) = 163.72, p<0.0001; time effect: F (2, 80) = 407.13, p<0.0001; treatment and time interaction: F (6, 80) = 11.06, p<0.0001; Fig. 6].

In experiment 6, sole ICV injection of the MTII (2.5 ng) or glutamate (75 nmol) had no effect on feeding behavior in FD3 neonatal broilers compared to the control group (P >0.05). Co-injection of the MTII + glutamate significantly decreased feed intake compared to glutamate or MTII alone [treatment effect: F (3, 80) = 439.52, p<0.0001; time effect: F (2, 80) = 376.08, p<0.0001; treatment and time interaction: F (6, 80) = 6.27; p<0.0001; Fig. 6].

**DISCUSSION**

To the best of our knowledge, this is the first report on the role of the glutamate with melanocortin and corticotrophin systems on feed intake in FD3 neonatal meat-type chicks. Based on the findings of the current study, dose dependent hypophagia was observed by ICV injection of the glutamate (75, 150 and 300nmol) in neonatal broiler chicken (figure 1). ICV injection...
of the metabotropic glutamate receptors antagonist increased food intake in broilers (Baghbanzadeh and Babapour, 2007). ICV injection of the NMDA receptor antagonist (DL-AP5) increased cumulative feed intake in FD₃ cockerels (Taati et al., 2011). Injection of NMDA and AMPA-kainite receptor antagonists into ventral pallidal and ventral striatal nuclei decreased feed intake in the pigeon (Da Silva et al., 2003). The effect of the glutamatergic system on feed intake is mediated via NMDA, AMPA and mGluR, receptors in FD₃, neonatal chicken (Torkzaban et al., 2017). It seems, glutamate metabotropic receptors have partial interaction with other neurotransmitters in feed intake regulation in broilers (Torkzaban et al., 2017). The mGluR₃ mechanically acts via phospholipase C activation which leads to the formation of the IP₃ and diacylglycerol, intracellular release of Ca²⁺ and stimulation of protein kinase C, while mGluR₁ and mGluR₂ coupled to adenylyl cyclase and cyclase respectively (Mortezaei et al., 2013).

Co-injection of the astressin-B + glutamate and astressin2-B + glutamate decreased glutamate-induced hypophagia in neonatal broiler chicken (figures 2 and 3). The interconnection is reported between CRF₁ and CRF₂ receptors with glutamatergic system. Activation of the CRF₁ and CRF₂ receptors have opposed actions on glutamatergic transmission in amygdala and the lateral septum mediolateral nucleus (Liu et al., 2004). Both CRF₁ and CRF₂ receptors are highly expressed in amygdala and the lateral septum mediolateral nucleus and influences neuronal properties (Liu et al., 2004). In the VTA, excitatory glutamatergic transmission is potentiated by CRF₁ modulation of the NMDA transmission (Liu et al., 2004). In the current study, co-injection of the glutamate + MC₃/MC₄ receptors antagonist decreased hypophagic effect of the glutamate in chicken (figures 4 and 5). In this regard, Lu et al., (2003) reported MC₃ and MC₄ receptors decrease feed intake in rats and nonhuman primates. Both CRF and MC₄ R mRNA are expressed in the PVN. MTII-induced plasma corticosterone was abolished by injection of the HS014 (0.25–1.0 nmol, selective MC₄ antagonist) (Lu et al., 2003). In MC₄−/− mice, because of the inability of MTII to suppress feed intake, MTII-induced anorexia mediates by the MC₄ R (Marsh et al., 1999).

The CRF has two side effects on glutamatergic synaptic transmission in the central nucleus of the amygdala which CRF₁ and CRF₂ receptors has inhibitory and facilitatory roles, respectively (Liu et al., 2011). Mice lacking CRF₁ receptors in glutamatergic neurons reduced anxiety and impaired neurotransmission in the hippocampus while increased anxiety-like behavior and reduced dopamine release in the prefrontal cortex (Inda et al., 2017). Under physiological conditions CRF₁ controlled dopaminergic and glutamatergic systems and could function in an antagonistic manner to retain adaptive responses to stressful situations in balance (Kratzer et al., 2013). Presynaptic glutamatergic neurotransmission increases by CRF in the central amygdala (Silberman & Winder, 2013). Extracellular glutamate levels increase after ICV injection of CRF into the central amygdala (Skorzewska et al., 2009). CRF, has a higher affinity for CRF which suggested CRF increases glutamatergic neurotransmission in the central amygdala via CRF2 even after CRF1 receptors become saturated (Silberman & Winder, 2013). Corticotropin-releasing factor neurons express the NMDA receptor and this protein is transported to intracellular locations in dendrites (Beckerman et al., 2013).

Co-injection of MC₃/MC₄ receptors agonist with NMDA glutamate receptors antagonist, decreased melanocortine-induced hypophagia in neonatal chicken (Ahmadi et al., 2017). Hypophagic effect of melanocortin is mediated by glutamatergic system in rats (Campos et al., 2015). ICV administration of the MTII into the NTS decreased feed intake and this effect was weakened by glutamatergic system (Carlos et al., 2015). Activation of the presynaptic MC₃ receptors in the central vagal afferent terminals by MTII reduces feed intake in rats (Campos et al., 2014). Glutamate release is required for phosphorylation of synapsin (I) in afferent vagal endings (Carlos et al., 2015). Synapsin (I) activation due to glutamate release is required for MTII-induced hypophagia in the rat (Carlos et al., 2015). Blockade of the glutamate receptor attenuated MTII-induced hypophagia in rats (Carlos et al., 2015). It seems, hypophagic effect of the melanocortin neurons acts by a mediatory role of the pro-opiomelanocortin (POMC) on the glutamate receptors (Dicken et al., 2012). Both POMC and MC₃ and MC₄ receptors have distribution on ARC and NTS which agouti related protein (AgRP) and neuropeptide Y (NPY) express (Gautron et al., 2010). The POMC neurons mediate glutamate release in the NTS which contribute to the reduction in food intake in the presence of MC₃ receptors agonists (Gautron et al., 2010). POMC and AgRP neurons are located proximate to each other parallel to the glutamate, MC₃ and MC₄ receptors in the ARC. Based on the literature, there was no previous report on interaction between glutamate...
One-day-old male-chickens (ROSS 308) n=264

Birds kept as flocks for 2 days until 5 days of old, then transferred into individual cages

Experiment 1
(each 4 groups within
11 replicates in each)

Experiment 2
(each 4 groups within
11 replicates in each)

Experiment 3
(each 4 groups within
11 replicates in each)

Experiment 4
(each 4 groups within
11 replicates in each)

Experiment 5
(each 4 groups within
11 replicates in each)

Experiment 6
(each 4 groups within
11 replicates in each)

ICV injection at 5 days old

Group (A) saline
Group (B) glutamate
Group (C) glutamate
Group (D) glutamate

Group (A) saline
Group (B) astressin-B
Group (C) glutamate
Group (D) astressin-B + glutamate

Group (A) saline
Group (B) astressin2-B
Group (C) glutamate
Group (D) astressin2-B + glutamate

Group (A) saline
Group (B) SHU9119
Group (C) glutamate
Group (D) SHU9119 + glutamate

Group (A) saline
Group (B) MCL0020
Group (C) glutamate
Group (D) MCL0020 + glutamate

Group (A) saline
Group (B) MTII
Group (C) glutamate
Group (D) MTII + glutamate

Then cumulative feed intake measured at 30, 60
and 120 minutes post ICV injection

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Food intake measured in each group

One-day-old male-chickens (ROSS 308) n=264

Birds kept as flocks for 2 days until 5 days of old, then transferred into individual cages

Experiment 1
(each 4 groups within
11 replicates in each)

Experiment 2
(each 4 groups within
11 replicates in each)

Experiment 3
(each 4 groups within
11 replicates in each)

Experiment 4
(each 4 groups within
11 replicates in each)

Experiment 5
(each 4 groups within
11 replicates in each)

Experiment 6
(each 4 groups within
11 replicates in each)

ICV injection at 5 days old

Group (A) saline
Group (B) glutamate
Group (C) glutamate
Group (D) glutamate

Group (A) saline
Group (B) astressin-B
Group (C) glutamate
Group (D) astressin-B + glutamate

Group (A) saline
Group (B) astressin2-B
Group (C) glutamate
Group (D) astressin2-B + glutamate

Group (A) saline
Group (B) SHU9119
Group (C) glutamate
Group (D) SHU9119 + glutamate

Group (A) saline
Group (B) MCL0020
Group (C) glutamate
Group (D) MCL0020 + glutamate

Group (A) saline
Group (B) MTII
Group (C) glutamate
Group (D) MTII + glutamate

Then cumulative feed intake measured at 30, 60
and 120 minutes post ICV injection

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Feed intake measured in each group

Food intake measured in each group
with melanocortin and corticotrophin systems on feed intake. So, we were not able to compare our results with it. In conclusion, these results suggested hypophagic effect of the glutamate mediates via CRF1/CRF2 and MC3/MC4 receptors in chickens. However, further investigation is required to elucidate the underlying cellular and molecular signaling pathways in the interconnections between glutamate with melanocortin and corticotrophin systems on food intake in neonatal chicks.

COMPLIANCE WITH ETHICAL STANDARDS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

INFORMED CONSENT

This manuscript does not contain any studies with human subjects performed by any of the authors.

HUMAN AND ANIMAL 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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