Role of Central Cannabinoidergic System on Ghrelin-Induced Hypophagia in Layer-Type Neonatal Chicken

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Abstract:

BACKGROUND: Feeding behavior is regulated via a complex network which interacts through diverse signals from central and peripheral tissues.

OBJECTIVES: The main purpose of the current study was to determine the role of central cannabinoidergic (CBergic) system on ghrelin-induced hypophagia in 3-h food deprived (FD3) neonatal chicken.

METHODS: In experiment 1, chicks were ICV injected with control solution, ghrelin (0.6 nmol), SR141716A (selective CB1 receptors antagonist, 6.25 µg) and ghrelin + SR141716A. In experiment 2, chickens received ICV injection of (A) control solution, ghrelin (0.6 nmol), AM630 (selective CB2 receptors antagonist, 1.25 µg) and ghrelin + AM630. In experiment 3, chickens were ICV injected with control solution, 2-AG (selective CB1 receptors agonist, 2µg), GSK1614343 (selective ghrelin receptors antagonist, 6 nmol) and 2-AG + GSK1614343. In experiment 4, the birds received control solution, CB65 (selective CB2 receptors agonist, 6.25 µg), CB65 + GSK1614343. Then the cumulative food intake was measured until 120 min post injection.

RESULTS: According to the results, ICV injection of the ghrelin, significantly decreased cumulative food intake ($P<0.05$). Co-injection of the ghrelin + SR141716A and/or ghrelin + AM630 significantly amplified ghrelin-induced hypophagia compared to control group ($P<0.05$). Hyperphagia observed by ICV injection of the 2-AG (2 µg) ($P<0.05$). Co-injection of the 2-AG + GSK1614343 increased food intake compared to control group ($P<0.05$). ICV injection of the CB65 (1.25 µg) significantly increased food intake in FD3 neonatal layer-type chicken ($P<0.05$). Also, co-injection of the CB65 + GSK1614343 significantly amplified cumulative food intake in FD3 neonatal layer-type chicken ($P<0.05$).

CONCLUSIONS: These results suggested ghrelin-induced hypophagia mediates via CB1 and CB2 receptors in neonatal layer-type chicken.

Keywords: Cannabinoid, Central food intake, Ghrelin, Neonatal layer-type chicken

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Introduction

Food intake and energy expenditure as a complex phenomenon is regulated by a complex neural system from central and peripheral signals (Skibicka et al. 2013). Based on neurobiological reports, gut–brain peptides such as ghrelin have regulatory role on appetite and reward (Engel et al. 2015) which in mammalian ghrelin is the only orexigenic hormone identified to date (Kalafateli et al. 2018). Circulating hormone ghrelin and the neural circuits in the central nervous system (CNS) have an important role in appetite control (Caldona Cano et al. 2012). Avian ghrelin is a 26-amino acid peptide which is identified in the stomach, brain and abdominal fat (Pingwen et al. 2011). Ghrelin has 3 subtypes of ghrelin receptors including S-R1a, GHS-R1aV (homologous to mammalian GHS-R1b) and GHS-R1tv which have been identified in broilers. Ghrelin provokes release of growth hormone through GH secretagogue receptors (GHS-R) (Engel et al. 2015). Systemic or Intracerebroventricular (ICV) injection of the ghrelin increases food intake in rat (Kaiya et al. 2013) while ghrelin inhibits feed intake in broiler (Saito et al. 2005; Zendehdel et al. 2012) and Japanese quail (Kitazawa et al. 2017). It has regulatory role on the mesolimbic reward system including ventral tegmental area (VTA) and the nucleus accumbens (NAc) which are involved in hedonic feeding (Skibicka et al. 2013).

The endocannabinoids (ECBs) have two receptors (CB1 and CB2). CB1 are receptors expressed abundantly in the CNS while CB2 receptors are identified in the peripheral nervous system (PNS), immune cells and tissues (Hassanpour et al. 2015). ICV injection of the CB agonists stimulated food intake in rodents while SR141716A (selective CB1 receptors antagonist) decreased food consumption in rat (Ierucka-Rybak et al. 2016). ICV administration of 2-AG (CB1 cannabinoid receptors agonist, 1 μg) or JWH015 (CB2 cannabinoid receptors agonist, 25 μg) amplified food intake in neonatal layer type chicken (Alizadeh et al. 2015) while CB2 receptors have orexigenic role in neonatal broilers (Emadi et al. 2011). However, Novoseletsky et al. (2011) reported intravenous injection of AM251 (inverse CB1 receptors agonist) leads to transient attenuation on food intake in meat-type chicken. Despite more than two decades passing from ghrelin discovery, its interaction with other neurotransmitters is not fully clarified yet (Zendehdel and Hassanpour, 2014). It is assumed an interaction exists between central ghrelin with other neurotransmitters such as dopamine (Skibicka et al. 2012), glutamate (Fuente-Martín et al. 2016) and CBergic systems. It is reported ICV injection of the CB receptor antagonist attenuates ghrelin-induced activation of the mesolimbic dopamine system in mice (Kalafateli et al. 2018). Ghrelin elevates hypothalamic ECB content in wild-type mice, but not in CB1 KO or rimonabant-treated mice (Lim et al. 2013). Additionally, CBs require the ghrelin receptor on ghrelin-induced cellular energy metabolism (Lim et al. 2013). So far, several researches have been done to determine central food intake regulation in both avian and mammals but these regulatory systems are not similar among them. For instance, in rodents, ghrelin has orexigenic effect (Kaiya et al. 2011) while it has anorexigenic role in domestic fowls (Saito et al. 2005; Zendehdel and Hassanpour 2014).
Therefore, the possible contribution of central ghrelin and CBergic system on food intake regulation will be employed in the layer industry to enhance hen’s productivity by manipulating appetite and lessening malnutrition concerns. So, the aim of the current study was to determine interaction of central CBergic system on ghrelin-induced hypophagia in neonatal layer-type chicken.

Material and Methods

Animals: A total of 176 one-day-old layer-type chickens (Hy-line) were purchased from a local hatchery. At 2 days of age, birds were randomly transferred to individual cages and kept at a temperature of 30 ± 1 °C with 50 ± 2 percent humidity (Olanrewaju et al. 2017). Birds were housed according to a completely randomized design. During the study birds had ad libitum access to fresh water and a starter diet containing 21% crude protein and 2850 kcal/kg of metabolizable energy (Animal Science Research Institute Co., Iran) (table). Three hours prior to the injections, birds were food deprived (FD3) but had free access to water. 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) and the current laws of the Iranian government for animal care.

Experimental Drugs: Drugs include ghrelin, SR141716A (a selective CB1 receptors antagonist), AM630 (a selective CB2 receptors antagonist), GSK1614343 (selective ghrelin receptors antagonist), 2-AG (2-Arachidonoylglycerol, a selective CB1 receptors agonist), CB65 (a selective CB2 receptors agonist) and Evans blue were purchased from Sigma Co. (Sigma, USA) and Tocris Co. (UK). Drugs except 2-AG were first dissolved in absolute dimethyl sulfoxide (DMSO) then diluted with 0.85% saline containing Evans blue at a ratio of 1/250. DMSO with this ratio does not have cytotoxic effect (Qi et al. 2008).

ICV injection procedures: In this study, 4 experiments designed to investigate interconnection of CBergic system with ghrelin on cumulative food intake in neonatal meat-type birds (each experiment includes 4 groups within 11 replicates in each group). Prior to each experiment, the chicks were weighed and based on their body weight divided into experimental groups so the average weight between treatment groups was as uniform as possible. ICV injection was applied using a microsyringe (Hamilton, Switzerland) without anesthesia according to the technique previously described by Davis et al. (1979) and Furuse et al. (1997) in which head of the birds was held with an acrylic device while the bill holder was 45° and calvarium parallel to the surface of table (Van Tienhoven and Juhasz, 1962). A hole was drilled in a plate in which the skull over the right lateral ventricle was immediately overlaid through this plate. A microsyringe was inserted into the right ventricle via the hole and tip of the needle penetrated 4 mm beneath the skin of the skull. It is revealed that, there is no injection-induced physiological stress using this method in neonatal chicks (Saito et al. 2005). Each chick received an ICV injection (with vehicle or drug solution) in a volume of 10 μL (Furuse et al. 1999). The control group received control solution (saline containing Evan’s blue 10 μL) (Furuse et al. 1999). Right away after injection, FD3 birds were returned to their individual cages and were supplied with fresh water and food (pre-weighed). Food consumption was calcu-
lated as a percentage of body weight (BW) (g/100g BW) to minimize impact of body weight on the amount of food intake. Each bird was used just once in each experimental group. At the end of the experiments, accuracy of placement of the injection in the ventricle was verified by presence of Evans blue followed by slicing the frozen brain tissue. All experimental procedures were done from 8:00 until 15:30.

**Feeding experiments:** In experiment 1, four groups of FD3 chicks received a dose of either the ICV injection of (A) control solution, (B) ghrelin (0.6 nmol), (C) SR141716A (6.25 µg) and (D) ghrelin + SR141716A. In experiment 2, FD3 chicks received ICV injections as follows: (A) control solution, (B) ghrelin (0.6 nmol), (C) AM630 (1.25 µg) and ghrelin + AM630. In experiment 3, fasted chickens were ICV injected with (A) control solution, (B) 2-AG (2µg), (C) GSK1614343 (6 nmol) and (D) 2-AG + GSK1614343. In experiment 4, the birds received (A) control solution, (B) CB65 (6.25 µg), (C) GSK1614343 (6 nmol) and (D) CB65 + GSK1614343. These doses of drugs were determined according to the previous and our pilot studies (Emadi et al. 2011; Novoseletsky et al. 2011; Zendehdel and Hassanpour, 2014).

**Statistical analysis:** Data is presented as mean ± SEM (standard error of the mean). Cumulative food intake (as percent of body weight) was analyzed by repeated measure two-way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). For treatment showing a main effect by ANOVA, means were compared by Tukey-Kramer test. \( P<0.05 \) was considered as significant difference between treatments.

**Results**

Effect of ICV injection of ghrelin, CB1 receptors antagonist and their combination on cumulative food intake in neonatal chickens is presented in Fig. 1. According to the results, ICV injection of the effective dose of the ghrelin, significantly decreased cumulative food intake in FD3 compared to control group (\( P<0.05 \)). ICV injection of sub effective dose of the SR141716A (6.25 µg) had no significant effect on food intake in FD3 birds (\( P>0.05 \)). Co-injection of the ghrelin + SR141716A significantly amplified ghrelin-induced hypophagia compared to control group (\( P<0.05 \)) (Fig. 1).

Effect of ICV injection of ghrelin, CB2 receptors antagonist and their combination on cumulative food intake in neonatal chickens is shown in Fig. 2. ICV injection of the effective dose of the ghrelin, significantly decreased cumulative food intake in FD3 compared to control group (\( P<0.05 \)). ICV injection of the sub effective dose of the AM630 (1.25 µg) had no significant effect on food intake in FD3 birds (\( P>0.05 \)). Co-injection of the ghrelin + AM630 significantly amplified hypophagic effect of the ghrelin compared to control group (\( P<0.05 \)) (Fig. 2).

Effect of ICV injection of selective ghrelin receptors antagonist, CB1 receptors agonist and their combination on cumulative food intake in neonatal chickens is presented in Fig. 1. Hyperphagia is observed by ICV injection of the 2-AG (2 µg) (\( P<0.05 \)). ICV injection of the GSK1614343 (6 nmol) had no significant effect on food intake in FD3 neonatal layer-type chicken (\( P>0.05 \)). Co-injection of the 2-AG + GSK1614343 increased food intake compared to control group (\( P<0.05 \)) (Fig. 3).
Table 1. Ingredient and nutrient analysis of experimental diet. 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 iodide 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, 0.36 g of folic acid, 1500 mg of choline chloride.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(%)</th>
<th>Nutrient analysis</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>52.85</td>
<td>ME, kcal/g</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>31.57</td>
<td>ME, kcal/g</td>
</tr>
<tr>
<td>Wheat</td>
<td>5</td>
<td>Crude protein (%)</td>
</tr>
<tr>
<td>Glutem meal, 61% CP</td>
<td>2.50</td>
<td>Linoleic acid (%)</td>
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<tr>
<td>Wheat bran</td>
<td>2.47</td>
<td>Crude fiber (%)</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.92</td>
<td>Calcium (%)</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>1.23</td>
<td>Available phosphorus (%)</td>
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<tr>
<td>Soybean oil</td>
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<td>Sodium (%)</td>
</tr>
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<td>Mineral premix</td>
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<td>Potassium (%)</td>
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<td>Vitamin premix</td>
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<td>Sodium bicarbonate</td>
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<td>Choline (%)</td>
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<td>Sodium chloride</td>
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<td>Calcium (%)</td>
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<td>DL-Methionine</td>
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<td>Lysine (%)</td>
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<td>Toxin binder</td>
<td>0.10</td>
<td>Methionine (%)</td>
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<td>L-Lysine HCl</td>
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<td>Methionine + cystine (%)</td>
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<td>Vitamin D3</td>
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<tr>
<td>Multi enzyme</td>
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<td>Tryptophan (%)</td>
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<tr>
<td></td>
<td></td>
<td>Valine (%)</td>
</tr>
</tbody>
</table>

Effect of ICV injection of selective ghrelin receptors antagonist, CB2 receptors agonist and their combination on cumulative food intake in neonatal chickens is shown in Fig. 4. ICV injection of the CB65 (1.25 µg) significantly increased food intake in comparison to the control group (P<0.05). ICV injection GSK1614343 (6 nmol) as sub effective dose, had no effect on feeding behavior in FD3 neonatal layer-type chicken (P>0.05). However, co-injection of the CB65 + GSK1614343 significantly amplified cumulative food intake in FD3 neonatal layer-type chicken (P<0.05) (Fig. 4).

Discussion

To the best of our knowledge, this is the first report on interaction of central CBergic system on ghrelin-induced hypophagia in neonatal layer-type chicken. Based on the results, ICV injection of the ghrelin significantly decreased cumulative food intake in FD3 neonatal layer-type chicken. ICV injection of the ghrelin decreases food intake in neonatal chicks (Saito et al. 2005). ICV injection of ghrelin (0.3, 1.1, 4.3 and 6.2 nmol) decreased food intake in 8-week-old broilers (Müller et al. 2015). ICV injection of the ghrelin (1 nmol) decreased food intake in Japanese quail (Kitazawa et al. 2017). ICV injection of the ghrelin increases food intake in laboratory animals (Kaiya et al. 2013). Injection of ghrelin increased in dopamine β-hydroxylase expression in nucleus tractus solitarius (NTS) (Date et al. 2006). The role of ghrelin is completely different among avian and mammalian. It is an orexigenic peptide in mammalian while
it is known as an anorexigenic neurotransmitter in chicken (Saito et al. 2005; Zendehdel and Hassanpour, 2014). In mammals, ghrelin-induced hyperphagia mediates via activating neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons. Ghrelin receptors are highly expressed in NPY than pro-opiomelanocortin (POMC) neurons in rat brain (Kaiya et al. 2013). Given the estimated 300 million years of evolutionary distance between mammals and avian, it is not surprising the discrepancy has been found in the central food intake and energy expenditure regulation (Novoseletsky et al. 2011).

According to the results, hyperphagia observed by ICV injection of the CB1 and CB2 receptors agonists, 2-AG and CB65,
respectively which was similar to mammals (Wiley et al. 2012; Ierucka-Rybak et al. 2016; Androvicova et al. 2017). However, the inconsistency between broiler and layers might be related to the differences in localization, affinity or expression of CB1 receptors (Emadi et al. 2011; Novoseletsky et al. 2011). Although CB2 receptors are primarily expressed in the cells and organs of the immune system, CB2-like protein has been isolated in the brain of neonatal chicks and disappears in adult chickens (Breivogel et al. 2018). CB receptors impress orexigenic effect by stimulation of NPY and blocking of the POMC neurons in the arcuate nucleus (D’Addario et al. 2014).

As observed, co-injection of the ghrelin + CB1 receptors antagonist amplified ghrelin-induced hypophagia. Co-injection of the ghrelin + CB2 receptors antagonist amplified hypophagic effect of the ghrelin. Co-injection of the CB1 receptors agonists + selective ghrelin receptors antagonist (GSK1614343) increased food intake. Also, co-injection of the CB2 receptors agonists + GSK1614343 amplified cumulative food intake in FD3 neonatal layer-type chicken. In a study, Senin et al. (2013) reported peripheral blockade of the CB1 receptor decreased food intake in food-deprived rat. This anorexigenic effect is likely a consequence of decreases in gastric ghrelin secretion. It is suggested CB1 receptor-mediated mechanism influences gastric ghrelin secretion and feeding behavior via mTOR pathway (Senin et al. 2013). In CB1-knockout mice ghrelin had no orexigenic effect. Pharmacological blockade of CB1 receptors inhibit signaling of the ghrelin on hypothalamic AMP-activated protein kinase (AMPK) activity (Lim et al. 2013). Ghrelin inhibits excitatory inputs of the CB1 receptors on the paraventricular nucleus (PVN) and this effect is abolished by ICV injection of CB1 antagonist in mice (Lim et al. 2013). So, CBergic signaling pathway is necessary for stimulatory effects of ghrelin on AMPK activity and food intake (Lim et al. 2013). Ghrelin exerts its hypothalamic effects via growth hormone secretagogue receptor type-1 and perhaps other receptors using this pathway to stimulate ECBS synthesis. Stimulatory effect of ghrelin on 2-AG blocked by rimonabant administration in mice. So, it seems interconnection exists between central CBergic and ghrelin neurons on food intake (Lim et al. 2013). In conclusion, these results suggested ghrelin-induced hypophagia mediates via CB1 and CB2 receptors in neonatal layer-type chicken. However, merit studies have been done on interaction of the ghrelin and CBergic in lab animal model but scarce information is present on interaction of the neurotransmitters on feeding behavior in avian. Further researches are needed to determine cellular and molecular mechanism(s) involved in interaction of the CB receptors on ghrelin-induced hypophagia in FD3 chicken.

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**Conflicts of interest**

The author declared no conflict of interest.

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نقطه سیستم کانابینوپدودزیک مرکزی بر هیپوفاژی ناشی از گرلین در جوجه‌های نژاد تخملگذار

متین مطالعه: افراد تغذیه‌ای از طریق مسیرهای پیچیده ای تنظیم می‌شود که توسط سیستم عصبی مرکزی و محیطی انجام می‌شود.
هدف مطالعه حاضر، بررسی نقش سیستم کانابینوپدودزیک مرکزی بر هیپوفاژی ناشی از گرلین در جوجه‌های نژاد تخملگذار است.

روش کار: در آزمایش 1، جوجه‌های 5 روزه تزریق داخل بطن مغزی محلول کنترل، گرلین (6/25 میکروگرم) و گرلین + SR141716A (آنتاگونیست انتخابی گیرنده‌های CB1) دریافت کردند. در آزمایش 2، جوجه‌های 5 روزه تزریق داخل بطن مغزی محلول کنترل، گرلین (6/25 میکروگرم) و گرلین + AM630 (آنتاگونیست انتخابی گیرنده‌های CB2) دریافت کردند. در آزمایش 3، جوجه‌های 5 روزه تزریق داخل بطن مغزی محلول کنترل، گرلین (6/25 میکروگرم) و گرلین + GSK1614343 (آنتاگونیست انتخابی CB1) و AG (آگونیست انتخابی CB2) دریافت کردند. در آزمایش 4، جوجه‌های 5 روزه تزریق داخل بطن مغزی محلول کنترل، گرلین (6/25 میکروگرم) و گرلین + CB65 (آنتاگونیست گیرنده‌های گرلین) دریافت کردند.

نتایج: جوجه‌های نژاد تخملگذار و جوجه‌های نژاد تخملگذار توزیع می‌باشد، بنابراین جوجه‌های تغذیه‌ای از طریق مسیرهای پیچیده تنظیم می‌شود.

واژه‌های کلیدی: کانابینوپدودزیک، انتخابی گیرنده‌های CB1 و CB2.