

# Interaction Between Endocannabinoid and Opioidergic Systems Regulates Food Intake in Neonatal Chicken

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Accepted: 23 February 2015 / Published online: 27 February 2015  
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**Abstract** Endocannabinoids and opiates have regulatory role in some physiological functions in mammals but their interaction(s) have not been studied in avian. This survey is designed to investigate interaction of these systems on feeding behavior in neonatal chickens. In experiment 1, chicken intracerebroventricular (ICV) injected with saline, DAMGO ( $\mu$ -opioid receptors agonist, 125 pmol), SR141716A ( $CB_1$  receptors antagonist, 6.25  $\mu$ g) and SR141716A + DAMGO. In experiment 2, saline, DAMGO, AM630 ( $CB_2$  receptors antagonist, 1.25  $\mu$ g) and DAMGO + AM630. Experiments 3–6 followed the procedure similar to experiments 1 and 2, except DPDPE ( $\delta$ -opioid receptors agonist, 40 pmol) and U-50488H ( $\kappa$ -opioid receptors agonist, 30 nmol) instead of DAMGO were used. In experiment 7, saline, Naloxone (opioid receptors antagonist, 5  $\mu$ g), 2-AG ( $CB_1$  receptors agonist, 2  $\mu$ g), Naloxone + 2-AG were used. Experiment 8 was similar to experiment 7, except CB65 ( $CB_2$  receptors agonist, 1.25  $\mu$ g) used instead of 2-AG. Cumulative food intake was recorded until 120 min post injection. Data provided that, ICV injection of DAMGO decreased food intake and its effect amplified by  $CB_1$  and  $CB_2$  receptors antagonist ( $P < 0.001$ ). DPDPE increased food intake and  $CB_2$  receptors antagonist blocked DPDPE-induced hyperphagia ( $P <$

0.001). U-50488H-induced feeding but its effect did not alter via  $CB_1$  and  $CB_2$  receptors antagonist ( $P > 0.05$ ). Hyperphagia-induced by  $CB_1$  and  $CB_2$  receptors agonist amplified by naloxone ( $P < 0.001$ ). Perhaps there is interaction between endocannabinoid and opioidergic systems on appetite regulation in chicken.

**Keywords** Cannabinoidergic · Opioidergic system · Food intake · Chicken

## Introduction

To date much progress has been done to identify mediatory effect of neurotransmitters on feeding behavior in mammals but food intake regulation in domestic poultry is not fully studied (Zendehdel and Hassanpour 2014). It is well documented that central feeding behavior is not regulated via a single neuropeptide and a wide distributed neural network interacts with diversity of neurotransmitters on feeding status (Irwin et al. 2008; Zendehdel et al. 2013a, 2014a). Cannabinoids (CBs) were originally characterized as psychoactive ingredients of Cannabis sativa plant or marijuana ( $\Delta^9$ -tetrahydrocannabinol, THC) (Novoseletsky et al. 2011). The isolation of CBs had important progress in neuropharmacology. To date two CB receptors have been identified which are belong to G-protein coupled receptors (GPCRs).  $CB_1$  receptors are mainly expressed in presynaptic terminals of inhibitory and excitatory nerves in the central nervous system (CNS) in both mammals and birds (Novoseletsky et al. 2011; Sharkey et al. 2014).  $CB_2$  receptors frequently found on cells and organs of the immune system in the peripheral nervous system (PNS) but also expressed in the brain (Onaivi et al. 2012). Pharmacological information revealed that CB receptors mainly

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expressed in prefrontal cortex (PFC), amygdala, septohippocampal system and arcuate nucleus (ARC) (Tambaro et al. 2013). It is suggested CBs have prominent role on feeding regulation which agonists stimulate appetite while antagonists have opposite effect in humans, rodents (Chen et al. 2006; Wiley et al. 2012) and poultry (Novoseletsky et al. 2011).

Opioids are known to be inhibitory neurotransmitters and three opioid receptor subtypes ( $\mu$ ,  $\kappa$  and  $\delta$ ) have been identified; all are members of the GPCR (Chen et al. 2006; Le Merrer et al. 2009). Opioid receptors are expressed in several parts of the hypothalamus, nucleus accumbens (NAcc), amygdala, ventral tegmental area (VTA) and nucleus tractus solitarius (NTS) which have regulatory role in pain perception, respiration, immune system and food intake (Barnes et al. 2006; Kozlov et al. 2013). Activation of central  $\mu$ -opioid receptors increases food consumption (Pritchett et al. 2010). Central injection of DAMGO and DPDPE ( $\mu$ - and  $\delta$ -receptors agonists, respectively) amplified eating in mammals (Taha, 2010; Kaneko et al. 2012). There is evidence that food-intake regulation mechanisms are different among mammals and birds (Zendehdel and Hassanpour, 2014). For instance, ICV injection of  $\mu$ -opioid receptors decreases food intake while  $\delta$ - and  $\kappa$ -opioid receptors increase food intake in chicks (Bungo et al. 2005). Interactions between opioids and other neurotransmitters have not been fully explored (Pritchett et al. 2010). It is suggested that neurological interaction exist between opioids and CBs which share a diversity of pharmacological properties e.g. hypothermia, sedation, hypotension and anti-nociception (Pickel et al. 2004). Increasing evidences suggest pro-dynorphins and pro-enkephalins regulate endocannabinoid level in animal's brain. These phenomena have supported the possible existence of functional linkage between two mechanisms which CBs and opioids mimic endogenous ligands, through the GPCRs, CB and opioid receptors (Manzanares et al. 2005). Pre-treatment with  $\Delta^9$ -THC increases enkephalins levels in the NAcc of mice (Ibegbu et al. 2011). It seems, these two systems interact via  $\mu$ - and  $\kappa$ -opioid receptors (Braidia et al. 2001; Ibegbu et al. 2011). Combined central injection of opioid receptor antagonist and CB<sub>1</sub> receptors agonists produced a synergistic inhibitory effect on feeding behavior in rat. However, direct molecular and cellular mechanism underlying this interconnection is not fully elicited (Chen et al. 2006).

In the ARC nucleus of hypothalamus, neuropeptide Y (NPY)/agouti related protein (AgRP) and pro-opiomelanocortin (POMC) and cocaine/amphetamine regulated transcript (CART) are the main sites which regulates appetite (Zendehdel et al. 2013b). Opioid receptors involve in orexigenic effect of NPY and AgRP neurons in the ARC (Barnes et al. 2006) where orexigenic effect of NPY mediates via  $\mu$ -opioid receptors in meat type chickens (Dodo

et al. 2005). Presumably, opioid and CB receptors in the NAcc interact with each other to modulate feeding (Skelly et al. 2010). No report was found on involvement of CBergic system on opioid-induced feeding behavior in neonatal chicken. So, the current research was conducted to specifically examine the role of ICV injection of CB receptors agonists and antagonists (CB<sub>1</sub> and CB<sub>2</sub>) on opioid receptors ( $\mu$ ,  $\delta$  and  $\kappa$ )-induced feeding in neonatal chickens.

## Materials and Methods

### Animals

To assume effect of CBergic system on opioid-induced feeding behavior, a batch of 384 1 day-old layer-type chickens (Seamorgh Co. Iran) were kept for 2 days as flocks. Then birds randomly allocated into their individual cages and kept at temperature of  $30 \pm 1$  °C with  $50 \pm 2$  % humidity until 5 days of age (Olanrewaju et al. 2006). During the study, animals provided mesh diet contains 21 % crude protein and 2850 kcal/kg of metabolizable energy (Animal Science Research Institute Co. Iran). Chickens had ad libitum access to food and fresh water during the study. Just 3-h prior the injections, animals were food deprived (FD<sub>3</sub>) but given free access to water. All birds received ICV injections 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) and the current laws of the Iranian government for animal care.

### Experimental Drugs

SR141716A (selective CB<sub>1</sub> receptors antagonist), AM630 (selective CB<sub>2</sub> receptors antagonist), DAMGO ( $\mu$ -opioid receptors agonist), DPDPE ( $\delta$ -opioid receptors agonist), U-50488H ( $\kappa$ -opioid receptors agonist), 2-AG (CB<sub>1</sub> receptors agonist), CB65 (CB<sub>2</sub> receptors agonist), Naloxone and Evans blue were purchased from Sigma Co. (Sigma, USA). Drugs except CB<sub>1</sub> receptors agonist (2-AG) were dissolved in 0.1 % Evans blue solution which was prepared in either 0.85 % saline. 2-AG first dissolved in absolute dimethyl sulfoxide (DMSO) then diluted with 0.85 % saline containing Evans blue at a ratio of 1/250. The control group in 2-AG treated experiment received DMSO containing saline diluted in mentioned ration.

### ICV Injection Procedures

Former to start experiments, chickens were weighed and based on their body weight distributed into treatment

groups. So, that the average weight between treatment groups was as uniform as possible. To assume interaction of CBergic and opioidergic systems on food intake in this study, eight experiments were designed, each include four treatment groups within 12 replicates in each group ( $n = 48$  birds per experiment). ICV injections were done using a microsyringe (Hamilton, Switzerland) without anesthesia in accordance to Davis et al. (1979) and Furuse et al. (1997). In this method, head of the chicken was held with an acrylic device while the bill holder was  $45^\circ$  and calvarium parallel to the surface of table (Van Tienhoven and Juhasz 1962). In a plate a hole was drilled which the skull over the right lateral ventricle immediately overlaid via this plate. In this technique microsyringe inserts into the right ventricle using the hole and tip of the needle penetrated 4 mm beneath the skin of the skull. There is no ICV injection induced physiological stress using this method in neonatal birds (Saito et al. 2005). At the end of the each experiment, accuracy of placement of the injection in the ventricle was verified by presence of Evans blue followed by slicing the frozen brain tissue. In each group, 12 birds received injections but just data of those individuals were used for analysis where dye was present into their lateral ventricle ( $n = 9$ –12 chickens/group). All experimental procedures were done from 9:00 a.m. until 1:00 p.m.

#### Food Intake Measurement Procedure

In this study, eight experiments were designed, each with four treatment groups: A, B, C and D, groups ( $n = 48$  in each experiment). In experiment 1, 3 h-fasted chickens were ICV injected as follows: (A) saline, (B) DAMGO ( $\mu$ -opioid receptors agonist, 125 pmol), (C): SR141716A (selective  $CB_1$  receptors antagonist, 6.25  $\mu$ g), (D): co-injection of SR141716A + DAMGO. Birds in experiment 2, injected with (A) saline, (B) DAMGO (125 pmol), (C): AM630 (selective  $CB_2$  receptors antagonist, 1.25  $\mu$ g), (D): co-injection of DAMGO +  $CB_2$  receptors antagonist. Birds in experiments 3–6 followed the procedure similar to experiment 1 and 2, except that chickens received ICV injection of DPDPE ( $\delta$ -opioid receptors agonist, 40 pmol) and U-50488H ( $\kappa$ -opioid receptors agonist, 30 nmol) instead of DAMGO. In experiment 7, (A) saline, (B) Naloxone (non-selective opioid antagonist, 5  $\mu$ g), (C): 2-AG (selective  $CB_1$  receptors agonist, 2  $\mu$ g), (D): co-injection of Naloxone + 2-AG. In experiment 8, followed the procedure similar to experiment 7 except that birds received ICV injection of CB65 (selective  $CB_2$  receptors agonist, 1.25  $\mu$ g) instead of 2-AG. All injections were done in a volume of 10  $\mu$ l. In all experiments, control group ICV injected with 10  $\mu$ l saline containing Evans blue. Each chick was injected once only. Right away after injection,

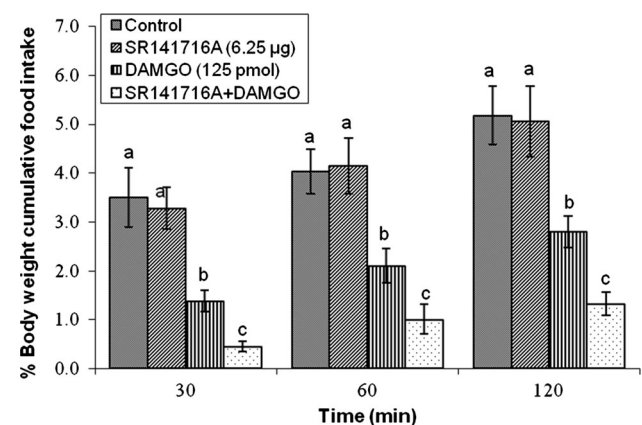
chickens were returned to their individual cages and provided ad libitum food (pre-weighed) and water. Cumulative food intake was recorded at 30, 60 and 120 post injection. Food consumption was calculated as percent of body weight (to adjust differences between body weights) (% BW). Injection dosages were calculated based on previous (Steinman et al. 1987; Bungo et al. 2004, 2005; Khan et al. 2009; Emadi et al. 2011) and our pilot studies (unpublished).

#### Statistical Analysis

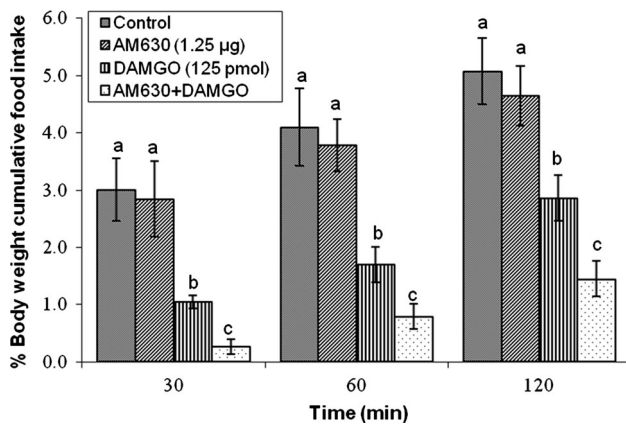
Cumulative food intake (% BW) was analyzed by two-way analysis of variance (ANOVA) for repeated measurement using SPSS 16.0 for Windows and is presented as mean  $\pm$  SEM. For treatment showing a main effect by ANOVA, means have compared by Duncan test.  $P < 0.05$  was considered as significant differences between treatments.

#### Results

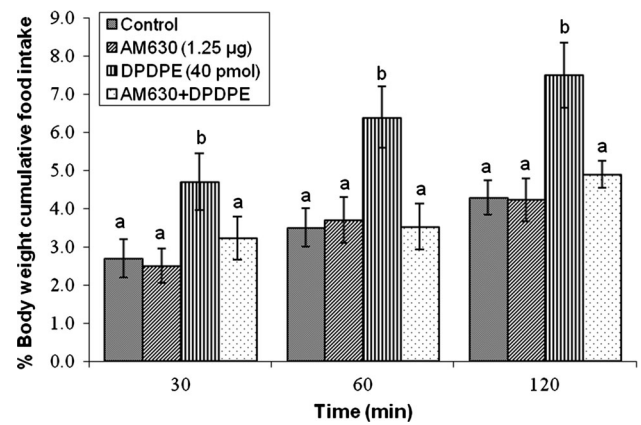
Effect of CBergic and opioidergic systems on cumulative food intake in  $FD_3$  neonatal layer-type chickens is presented in Figs. 1, 2, 3, 4, 5, 6, 7, and 8. According to the results, ICV injection of sub effective dose of selective  $CB_1$  (SR141716A, 6.25  $\mu$ g) and  $CB_2$  (AM630, 1.25  $\mu$ g,) receptors antagonists had no significant effect on food intake (% BW) compared to control group in experiments 1–6 ( $P > 0.05$ ). So, in this study we used from sub effective dose for  $CB_1$  and  $CB_2$  receptors antagonists.



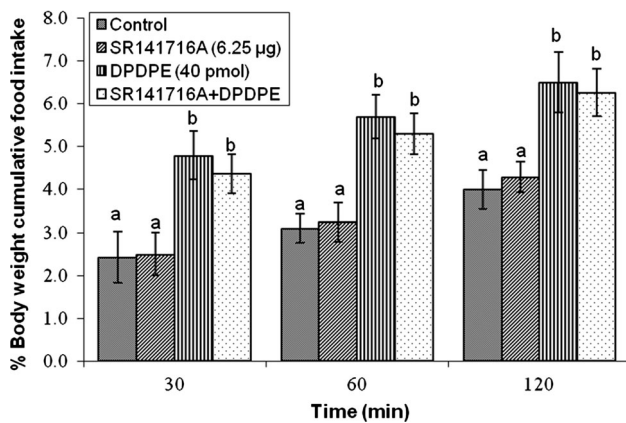
**Fig. 1** Effect of ICV injection of SR141716A (6.25  $\mu$ g), DAMGO (125 pmol), and their combination on cumulative food intake (% BW) in neonatal chickens. SR141716A: selective  $CB_1$  receptors antagonist, DAMGO:  $\mu$ -opioid receptors agonist. There are significant differences between groups with different superscripts in a column (a, b and c;  $P < 0.05$ )



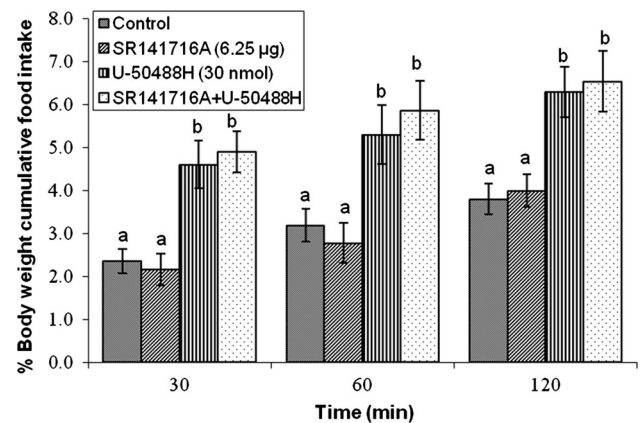
**Fig. 2** Effect of ICV injection of AM630 (1.25 µg), DAMGO (125 pmol), and their combination on cumulative food intake (% BW) in neonatal chickens. AM630: selective CB<sub>2</sub> receptors antagonist, DAMGO: µ-opioid receptors agonist. There are significant differences between groups with different superscripts in a column (*a*, *b* and *c*;  $P < 0.05$ )



**Fig. 4** Effect of ICV injection of AM630 (1.25 µg), DPDPE (40 pmol) and their combination on cumulative food intake (% BW) in neonatal chickens. AM630: selective CB<sub>2</sub> receptors antagonist, DPDPE (δ-opioid receptors agonist). There are significant differences between groups with different superscripts in a column (*a* and *b*;  $P < 0.05$ )



**Fig. 3** Effect of ICV injection of SR141716A (6.25 µg), DPDPE (40 pmol) and their combination on cumulative food intake (% BW) in neonatal chickens. SR141716A: selective CB<sub>1</sub> receptors antagonist, DPDPE (δ-opioid receptors agonist). There are significant differences between groups with different superscripts in a column (*a* and *b*;  $P < 0.05$ )



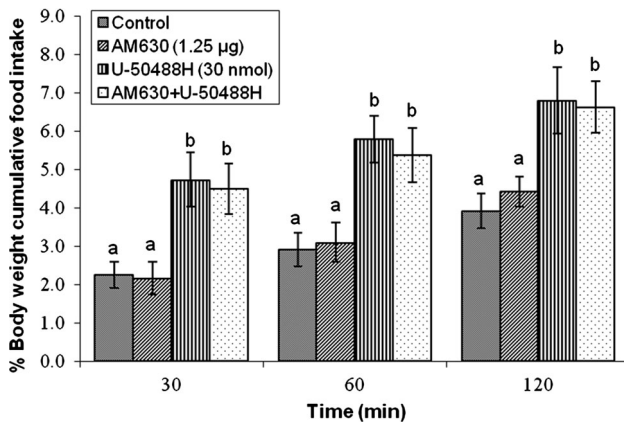
**Fig. 5** Effect of ICV injection of SR141716A (6.25 µg), U-50488H (30 nmol) and their combination on cumulative food intake (% BW) in neonatal chickens. SR141716A: selective CB<sub>1</sub> receptors antagonist, U-50488H (κ-opioid receptors agonist). There are significant differences between groups with different superscripts in a column (*a* and *b*;  $P < 0.05$ )

As seen, ICV injection of DAMGO (µ-opioid receptors agonist, 125 pmol) significantly decreased food intake compared to control group until 120 min post injection [ $F(1,34) = 157.01$ ,  $P < 0.001$ ]. The dose of DAMGO was selected based on our pilot study, because it was found to induce a decrease in food intake without affecting other non-ingestive behavioral parameters such as sedation. Also, co-administration of SR141716A and DAMGO amplified hypophagic effect of DAMGO in chickens [ $F(1,34) = 190.72$ ,  $P < 0.001$ ] (Fig. 1).

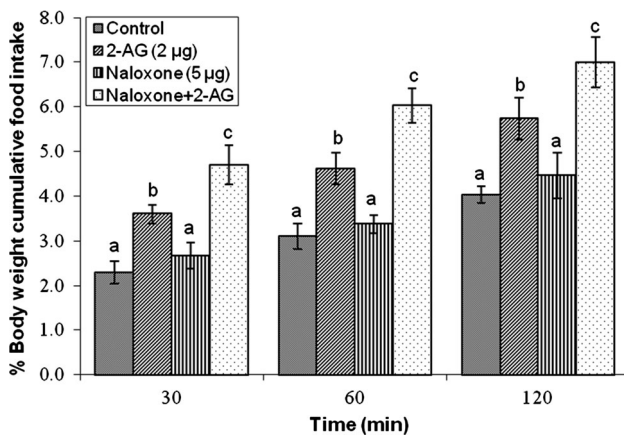
Effect of ICV injection of AM630 (1.25 µg, selective CB<sub>2</sub> receptors antagonist) and DAMGO on cumulative food intake (% BW) in FD<sub>3</sub> neonatal layer type chickens is

presented in Fig. 2. According to the data, there was no significant effect on food intake after ICV injection of CB<sub>2</sub> receptors antagonist (1.25 µg) [ $F(1,34) = 4.701$ ,  $P > 0.05$ ]. Furthermore, ICV injection of 125 pmol of DAMGO had hypophagic effect compared to control group ( $P < 0.001$ ). Co-administration of AM630 and DAMGO amplified hypophagic effect of DAMGO in neonatal layer type chickens compared to control group [ $F(1,34) = 140.02$ ,  $P < 0.001$ ].

According to the results of experiment 3, DPDPE (40 pmol) significantly increased food intake in chickens compared to control group [ $F(1,34) = 181.47$ ,  $P < 0.001$ ] and hyperphagic effect of DPDPE (40 pmol) was not significantly attenuated by co-injection of DPDPE and



**Fig. 6** Effect of ICV injection of AM630 (1.25 µg), U-50488H (30 nmol) and their combination on cumulative food intake (% BW) in neonatal chickens. AM630: selective CB<sub>2</sub> receptors antagonist, U-50488H (κ-opioid receptors agonist). There are significant differences between groups with different superscripts in a column (*a* and *b*;  $P < 0.05$ )

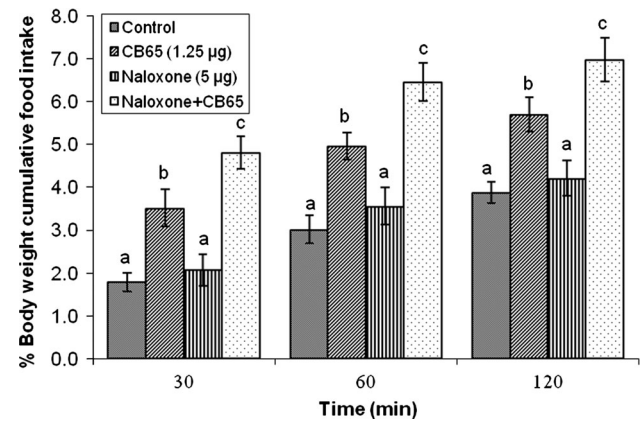


**Fig. 7** Effect of ICV injection of 2-AG (2 µg), Naloxone (5 µg) and their combination on cumulative food intake (% BW) in neonatal chickens. 2-AG: CB<sub>1</sub> receptors agonist, Naloxone: non-selective opioid receptors antagonist. There are significant differences between groups with different superscripts in a column (*a*, *b* and *c*;  $P < 0.05$ )

selective CB<sub>1</sub> receptors antagonist) [ $F(1,34) = 1.508$ ,  $P > 0.05$ ] (Fig. 3). Likewise, hyperphagic effect of DPDPE (40 pmol) significantly blocked by co-administration of DPDPE + AM630 (selective CB<sub>2</sub> receptors antagonist) [ $F(1,34) = 195.02$ ,  $P < 0.0001$ ] (Fig. 4).

As seen in experiment 5, U-50488H (κ-opioidergic receptors agonist, 30 nmol) significantly increased food intake but hyperphagic effect of U-50488H did not change by co-administration of U-50488H + SR141716A (CB<sub>1</sub> receptors antagonist) compared to control group in birds [ $F(1,34) = 6.739$ ,  $P > 0.05$ ] (Fig. 5).

According to the obtained data from experiment 6, the significant reinforced effect of U-50488H on food intake was similar to co-injection of U-50488H and AM630



**Fig. 8** Effect of ICV injection of CB65 (1.25 µg), Naloxone (5 µg) and their combination on cumulative food intake (% BW) in neonatal chickens. CB65: CB<sub>2</sub> receptors agonist, Naloxone: non-selective opioid receptors antagonist. There are significant differences between groups with different superscripts in a column (*a*, *b* and *c*;  $P < 0.05$ )

(selective CB<sub>2</sub> receptors antagonist) [ $F(1,34) = 4.006$ ,  $P > 0.05$ ] (Fig. 6).

In this study, food intake significantly increased via CB<sub>1</sub> receptors agonist (2-AG, 2 µg) compared to control group [ $F(1,34) = 143.18$ ,  $P < 0.001$ ]. Also, Naloxone (non-selective opioid antagonist, 5 µg) had no effect on food intake while co-administration of 2-AG + Naloxone significantly amplified hyperphagic effect of CB<sub>1</sub> receptors agonist [ $F(1,34) = 157.013$ ,  $P < 0.001$ ] (Fig. 7).

In experiment 8, administration of CB65 (CB<sub>2</sub> receptors agonist, 1.25 µg) improved food intake compared to control group in chicken [ $F(1,34) = 131.64$ ,  $P < 0.001$ ] and its effect augmented via co-administration of CB65 + Naloxone [ $F(1,34) = 124.84$ ,  $P < 0.001$ ] (Fig. 8).

## Discussion

The present survey provides support for physiologic relevant interactions between CBergic and opioidergic systems on food intake in FD<sub>3</sub> neonatal layer-type chickens. Despite psychoactive constituent of cannabis *sativa* (marijuana), Δ<sup>9</sup>-THC for the first time isolated in 1964 and specific CB receptors identified in 1980 but role of CBergic system is not fully elicited. To date several researches have done on role of CBergic system. Now evidences imply that endocannabinoids include in several behavior and emotional states, such as food intake, pain, anxiety and go fourth (López 2010). In the CNS, endocannabinoids produced from arachidonic acid on the cell membranes. CB<sub>1</sub> receptor is identified in the terminals of central, peripheral neurons and glial cells; however they are expressed in the peripheral tissue (D'Addario et al. 2014). It is reported CBergic system modulates food intake in neonatal broilers

(Emadi et al. 2011). ICV injection of low to mid-dose of SR141716A (selective CB<sub>1</sub> receptors antagonist) or AM251 (CB<sub>1</sub> cannabinoid receptors inverse agonist) has anorectic effect in rat (Chen et al. 2006). In this study, we used sub-effective dose of antagonists which blocks receptor without effect on food intake to assay possible interaction(s) of CBergic system on opioidergic-induced food intake.

μ-opioid receptors impress orexigenic effect on feeding behavior through nucleus accumbens (NAc) and NTS in rodents (Browning et al. 2006; Fichna et al. 2007; Zheng et al. 2007). Central appetite regulatory mechanisms in chicken are somewhat dissimilar than mammals. Controversial results exist about the effect of μ-opioid receptors on food intake in chickens. It is reported DAMGO inhibits feeding behavior in neonatal broiler (Bungo et al. 2004, 2005). In comparison, central μ-opioid receptors have orexigenic role in broilers (Khan et al. 2009). Our results revealed μ-opioid receptors have anorexigenic effect in layer-type chicken. Discrepancy between layer and broiler may be ascribed to genetic selection (Bungo et al. 2004; Zendejdel et al. 2014b) where genetic selection for growth in broilers altered their brain appetite regulation centers and/or pathways (Denbow 1994).

This study provides the first demonstration of hypophagia induced by central administration of μ-opioid receptors agonist mediates by selective CB<sub>1</sub> and CB<sub>2</sub> receptors antagonists in chicks (Fig. 1). CB<sub>1</sub> receptors in high density are expressed on presynaptic nerve terminals of both inhibitory and excitatory nerves, based on the brain region (Sharkey et al. 2014). CB<sub>1</sub> densities are identified in the amygdala and hypothalamus. CB<sub>2</sub> predominantly found in the peripheral tissues like spleen and leukocytes (López 2010). Interestingly, CB<sub>2</sub> is also present in the brain (D'Addario et al. 2014). Cannabinoid agonists may activate a comparative greater number of G-proteins per occupied receptor in these brain regions (López 2010).

Numerous hypotheses have been postulated to explain the mechanisms underlying the interaction between CBergic and μ-opioid receptors. Anatomical evidence suggesting both receptors involved in feeding regulation centers in the brain (Chen et al. 2006). In keeping with these observations, orexigenic mediator, NPY, increase via CB<sub>1</sub> receptors. CBergic system interacts by several anorexigenic and orexigenic neurotransmitters in the hypothalamus in regulation of energy expenditure and food intake (D'Addario et al. 2014). Both types of receptors are coupled to adenylyl cyclase activity, decrease in cAMP production through activation of G<sub>i</sub> proteins (Chen et al. 2006). Postsynaptic depolarization increase endocannabinoid synthesis binds to CB receptors on the presynaptic membrane and activates G protein, inhibits Ca<sup>2+</sup> influx and terminates to decrease neurotransmitter release (López 2010). CB<sub>1</sub> and μ-opioid

receptor interacts in the NAcc through a mechanism involving G<sub>α<sub>i</sub>inhibitory3</sub> protein (G<sub>α<sub>i3</sub></sub>) (Yao et al. 2006). It is reported food intake increases via μ-opioid receptor in rat and co-injection of naloxone (μ-opioid receptor antagonist) and selective CB<sub>1</sub> receptor antagonist suppressed food intake (Kirkham and Williams 2001). Perhaps, a systemic cannabinoid–opioid interaction may exist on appetite regulation (Chen et al. 2006). There are differences in the activities of neurotransmitters such as ghrelin (Zendejdel and Hassanpour, 2014), leptin and adiponectin (Novoseletsky et al. 2011) on feeding behavior regulation between mammals and avian. Suggested mechanism for rewarding effect of CBergic system on μ-receptor-induced feeding is that CBergic system mediates endogenous opioid systems via dopaminergic system (Braidia et al. 2001; Solinas and Goldberg, 2005) where mesolimbic incentive dopaminergic pathways are modulated by endocannabinoids and opioidergic reward circuits. So, these actions underlie the orexigenic potency of CBs (Bodnar 2012). We think, further researches needed to identify accuracy of this hypothesis in birds.

In this study, DPDPE (δ-opioid receptors agonist) significantly reinforced cumulative food intake. It is reported that following 12-h food deprivation, a peripheral injection of AM630 (selective CB<sub>2</sub> receptors antagonist) increased food intake in rat (Onaivi et al. 2008). Perhaps, CB<sub>2</sub> receptors act on the immune system and indirectly regulate feeding behavior by alter in the digestive system function (Emadi et al. 2011). As observed, co-administration of δ-opioid receptor agonist and CB<sub>1</sub> receptors antagonist was not able to diminish hyperphagic effect of DPDPE while this effect minimized via CB<sub>2</sub> receptors antagonist. δ-opioid receptor agonist elevates food intake in neonatal meat-type chicken (Bungo et al. 2004; Yanagita et al. 2008; Khan et al. 2009) and rats (Levine 2006; Kaneko et al. 2012). In normal physiological conditions, central prostaglandins D<sub>2</sub> (PGD<sub>2</sub>) and NPY system play deniable role in appetite and this interaction is via opioidergic system. Central injection of naltrindole (δ-opioid receptors agonist) activates cyclooxygenase-2 (COX-2) which terminates to produce PGD<sub>2</sub>, act on its specific receptor, DP<sub>1</sub> (GPCR subtype) and stimulates NPY level via DP<sub>1</sub>-possessing NPY neurons in the CNS. Presumably, δ-opioid-induced orexigenic activity is mediated via PGD<sub>2</sub>–NPY system (Kaneko et al. 2012). Hypothalamic endocannabinoid is necessary for orexigenic effect of NPY (Di Marzo et al. 2001). However, less information exist on interaction of CBergic system on δ-opioid-induced food intake. Stimulation of CB<sub>2</sub> receptors suppresses microglial activation. Perhaps this effect mediates through microglia. There is no evidence for expression or function of CB<sub>2</sub> receptors in the microglia and neurons in chicken (Emadi et al. 2011). In a sole study, Fowler et al. (2001) reported CB<sub>2</sub>-like protein exists in CNS of neonatal

chick and it disappears in adult chicken. We think much more research needs to clarify role of CB<sub>2</sub> receptors on feeding behavior in layer-type chicken. As seen, hyperphagic effect of U-50488H ( $\kappa$ -opioid receptors agonist) was not attenuated via both CB<sub>1</sub> or CB<sub>2</sub> receptor antagonists. It is reported that central injection of U-50488H elicits hyperphagia in broiler chickens (Bungo et al. 2004). Presumably,  $\kappa$ -opioid induced food intake is not mediated via CBergic system in layer-type chicken.

Of particular interest of present study was that co-administration of CB<sub>1</sub> or CB<sub>2</sub> agonist with naloxone significantly amplified hyperphagic effect of CB<sub>1</sub> receptor agonist. It is reported CBergic-induced food intake is attenuated by naloxone in rat where co-injection of naloxone and CB<sub>1</sub> receptors antagonist synergistically depress food intake in rat (Williams and Kirkham 2002). On the other hand, central injection of naloxone (10  $\mu$ g) elevated food intake in neonatal layer-type chicken (Steinman, et al. 1987). Interestingly, peripheral but not central administration of naloxone attenuated food intake in domestic fowl (McCormack and Denbow 1990). Naloxone attenuated food consumption through production of malaise or impairment of motor function where high levels of naloxone lead to overt symptoms of illness, discomfort or motor problems (McCormack and Denbow 1990). In the present study, we used sub-effective dose of naloxone (5  $\mu$ g) (Steinman et al. 1987) which blocked the opioid receptors without effect on food intake to investigate opioid receptors interconnection with CBergic receptors (Soria-Gomez et al. 2007). Naloxone precipitates opioid-like withdrawal syndrome in CB-dependent mice (Ghozland et al. 2002). Opioid and CB receptors in the NAcc interact with each other to modulate feeding (Skelly et al. 2010). Therefore, presumably neurotransmitter release not only modulates through CBergic system but it may also influence the expression of feeding related neuropeptides (Emadi et al. 2011). Based on literature, food intake diminished via  $\mu$ -opioid receptors in chicken which is dissimilar to rodents (Bungo et al. 2004). In this study, food intake increased with co-administration of naloxone and CB<sub>1</sub> and CB<sub>2</sub> receptors agonists in birds. To our knowledge we think blockade of opioidergic receptors might amplify hyperphagic effect of CBergic system in chicks. Most research on central food intake regulation has done in rat models whereas considering few investigations done in birds. Actually, there was no similar research to compare our results on mediatory effect of naloxone on cannabinoid-induced food intake as poultry model. It is reported, central role of opioidergic system is somewhat different in avian and mammalian (Zendehdel and Hassanpour, 2014). These controversial data might be the consequence of injection methods and species difference (Meade and Denbow 2001). To conclude new finding of current study can be used as base information on regulatory role of CBs on effect

of opioidergic system on food intake in neonatal chicken. Further research required to clarify any direct interaction of cellular and molecular signaling pathways in the interconnection between CBergic and opioidergic systems on feeding behavior in avian.

**Conflict of interest** Morteza Zendehdel, Shahin Hassanpour, Vahab Babapour, Saeed Charkhkar and Mahshid Mahdavi declare that they have no conflict of interest.

**Human and Animal Rights** All experiments executed in according to the Guide for the Care and Use of Laboratory Animals and approved by the institutional animal ethical committee.

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

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