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ORIGINAL ARTICLE

The interaction of central nitrergic and GABAergic systems on food intake in neonatal layer-type chicks

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Abstract Most physiological behaviors such as food intake are controlled by the hypothalamus and its nuclei. It has been demonstrated that injection of the paraventricular nucleus of the hypothalamus with nitric oxide (NO) donors elicited changes in the concentration of some amino acids, including GABA. Also, central nitrergic and GABAergic systems are known to provide inputs to the paraventricular nucleus and are involved in food intake control. Therefore, the present study examines the probable interaction of central nitrergic and GABAergic systems on food intake in neonatal layer-type chicks. The results of this study showed that intracerebroventricular (ICV) injection of L-arginine (400 and 800 nmol), as a NO donor, significantly decreased food intake ($P < 0.001$), but ICV injection of Nω-Nitro-L-arginine methyl ester (L-NAME) (200 and 400 nmol), a NO synthesis inhibitor, increased food intake ($P < 0.001$). In addition, the orexigenic effect of gaboxadol (0.2 µg), a $GABA_A$ agonist, was significantly attenuated in ICV co-injection of L-arginine (200 nmol) and gaboxadol (0.2 µg) ($P < 0.001$), but it was significantly amplified in ICV co-injection of L-NAME (100 nmol) and gaboxadol (0.2 µg) ($P < 0.001$). On the other hand, the orexigenic effect of baclofen (0.2 µg), a $GABA_B$ agonist, did not change in ICV co-injection of L-arginine (200 nmol) or L-NAME (100 nmol) with baclofen (0.2 µg) ($P > 0.05$). Also, the hypophagic effect of L-arginine (800 nmol) was significantly amplified in ICV co-injection of picrotoxin (0.5 µg), a $GABA_A$ antagonist, or CGP54626 (21 ng), a $GABA_B$ antagonist, with L-arginine (800 nmol) ($P < 0.001$). These results probably suggest an interaction of central nitrergic and GABAergic systems on food intake in neonatal layer-type chicks and $GABA_A$ receptors play a major role in this interaction.

Keywords NO · GABA · Food intake · Neonatal layer-type chick

Introduction

Numerous studies over the past several decades have shown that complex neurochemical pathways in different parts of the brain regulate appetite and food intake in animals (Alimohammadi et al. 2015). Nitric oxide (NO) is a gaseous neurotransmitter in the brain and is made from L-arginine by nitric oxide synthase (NOS) (Morris 2004). NO participates in several physiologic functions such as regulation of local cerebral blood flow during hypoxia (Takuwa et al. 2010), muscle repair and regeneration following repetitive eccentric contractions (Culotta and Koshland 1992) and food intake control (Morris 2004). Intracerebroventricular (ICV) injection of Nω-Nitro-L-arginine methyl ester (L-NAME), NOS inhibitor, decreased food intake in rats (De Luca et al. 1995) and avian species (Choi et al. 1994) and NO is recognized as an orexigenic molecule. On the other hand, some studies in neonatal chicks showed contrary results and L-NAME stimulated food intake (Alimohammadi et al. 2015; Hassanpour et al. 2015) and NO

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inhibited food intake in neonatal chicks (Khan et al. 2008). It is noteworthy that central nitrergic system is more active on food intake in layer-type chicks than meat-type chicks. Thus, NO is likely to play different roles in food intake regulation beyond the animal species (Khan et al. 2007).

ϒ-Aminobutyric acid (GABA), the most widely distributed inhibitory neurotransmitter in the vertebrate central nervous system (Sivilotti and Nistri 1991), is found in high concentrations in brain areas known to be involved in food intake control (Decavel and Van den Pol 1990). GABA exerts its effects by acting on two distinct receptors including the bicuculline-sensitive $GABA_A$ receptor and the bicuculline-insensitive $GABA_B$ receptors (Stratford and Wirtshafter 2013). Functionally, $GABA_A$ and $GABA_B$ receptors appear to be located both pre- and post synaptically (Tellez et al. 2012). In avian species, it is reported ICV injection of a $GABA_A$ receptor agonist (muscimol) increased food intake in turkeys (Denbow 1991) and meat-type chicks (Jonaidi et al. 2002; Zendehdel et al. 2009), but ICV injection of a $GABA_B$ receptor agonist (baclofen) had no effect on food intake in meat-type chicks (Jonaidi et al. 2002). In layer-type chicks, ICV injection of all GABA agonists induced hyperphagia (Bungo et al. 2003). It seems that central GABAergic system is more active on food intake in layer-type chicks than meat-type chicks.

Several reports showed that anatomical (Valtschanoff et al. 1993) and physiological (Pajolla et al. 2009) interactions exist between central nitrergic and GABAergic systems and pharmacological evidence showed a functional interaction between $GABA_A$ receptor activity and NO synthesis in the brain (Paul et al. 2001). Moreover, NO-GABA modulation on autonomic outflow was shown in the paraventricular nucleus (PVN) of the hypothalamus (Watkins et al. 2009) and microinjection or perfusion of the PVN with NO donors elicited changes in the concentrations of some amino acids, including GABA (Horn et al. 1994). In addition, central nitrergic and GABAergic systems are known to provide inputs to the PVN and are involved in food intake control. Thus, it is possible to assume an interaction between central intrergic and GABAergic systems on food intake and this study examines the interaction of these two central systems on food intake in neonatal layer-type chicks.

Materials and methods

Animals

One-day-old layer chicks were purchased from a local hatchery (Morghak Company, Tehran, Iran). The chicks were maintained in stabilizing electric heated batteries at a temperature of 32 °C ± 1, kept at 40–50 % relative humidity and housed in continuous lighting condition. They were provided with water and a commercial starter diet (containing 21 % crude protein and 2850 kcal/kg metabolizeable energy) (Chineh Co, Tehran, Iran) ad libitum. The chicks were kept as flock for 3 days, and then were placed in individual cages with free access to food and water. The experiments were started when the chicks were 5 days old. Food was deprived 3 h prior to ICV injection (FD_3) in all experiments. The chicks were maintained in accordance with the recommendations of the National Research Council.

Experimental drugs

All drugs were purchased from Sigma Co. (Sigma, USA). L-Arginine hydrochloride (NO donor; water soluble), Nω-Nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor; water soluble), gaboxadol (a $GABA_A$ agonist; soluble in Dimethyl sulfoxide: DMSO), picrotoxin (a $GABA_A$ antagonist; DMSO soluble), Baclofen (a $GABA_B$ agonist; water soluble) and CGP54626 (a $GABA_B$ antagonist; DMSO soluble). The drugs were dissolved in a 0.1 % Evans Blue solution, which was prepared in either 0.85 % saline or first dissolved in absolute DMSO and then diluted with saline at a ratio of 1/1250 (0.08 % DMSO). DMSO with this ratio does not have cytotoxic effect (Blevins et al. 2002; Qi et al. 2008). Saline containing Evans Blue with or without DMSO was also used as the control solution.

ICV injection

Prior to each experiment, the chicks were weighed and based on their body weight were divided into experimental groups. Therefore, the average weight among the groups was made as uniform as possible. Eight experiments were conducted and each experiment had four treatment groups ($n = 12$ per group). ICV injection was accomplished by a microsyringe without anesthesia according to Davis et al. (1979) and Furuse et al. (1997). Briefly, head of the alert chick was held with an acrylic device in which the bill holder was 45° and the calvarium was parallel to the surface of the table as described by Van Tienhoven and Juhasz (1962). A hole was drilled in a plate. This plate was overlaid on the skull immediately over the right lateral ventricle. Then a microsyringe was inserted into the ventricle through the hole and the test solution was injected. Only 4 mm below the skin of the skull was penetrated by the top of the needle. The procedure does not cause physiological stress in neonatal chicks (Furuse et al. 1999). At the end of the experiments, the chicks were decapitated by guillotine, and location of the injection site was verified. Only those data from individuals having dye (Evans Blue) present

in their lateral ventricle were used for statistical analysis (n = 9–11). The volume of each injection was 10 µL. After injection, the chick was immediately returned to its cage and fresh food and water were supplied. Cumulative food intake (gr) was measured at 30, 60 and 120 min after the injection and the time course of food consumption was selected from previous studies (Alimohammadi et al. 2015; Jonaidi et al. 2012). Food consumption was expressed as a percentage of body weight to adjust body weight differences. In this study, the mean volume of food consumption before correction by the percent of body weight was 0.46–3.5 g and average body weight of the chicks was 40–50 g.

Feeding experiments

Experiments 1 and 2 were designed to examine the effect of ICV injection of different doses of L-arginine (200, 400 and 800 nmol) and L-NAME (100, 200 and 400 nmol) on food intake in FD_3 neonatal layer-type chicks, respectively. In experiment 3, each treatment group received either control solution, L-arginine (200 nmol), gaboxadol (0.2 µg) or a co-injection of L-arginine (200 nmol) and gaboxadol (0.2 µg). In experiment 4, each treatment group received either control solution, L-NAME (100 nmol), gaboxadol (0.2 µg) or a co-injection of L-NAME (100 nmol) and gaboxadol (0.2 µg). Experiments 5 and 6 were similar to experiments 3 and 4, respectively, just baclofen (0.2 µg) was used instead of gaboxadol (0.2 µg). In experiment 7, each treatment group received either control solution, L-arginine (800 nmol), picrotoxin (0.5 µg) or a co-injection of L-arginine (800 nmol) and picrotoxin (0.5 µg). Experiment 8 was analogous to experiment 7, except CGP54626 (21 ng) was used instead of picrotoxin (0.5 µg). Experiments 3, 4 and 7 were designed to examine the interaction of central nitrergic system and−− $GABA_A$ receptors on food intake in neonatal layer-type chicks. Also, experiments 5, 6 and 8 examine the interaction of central nitrergic system and $GABA_B$ receptor on food intake in neonatal layer-type chicks. In the co-injections, both substances were administered in a unique injection. Drug doses were selected based on previous studies (Bungo et al. 2003; Takagi et al. 2003) and pilot studies (unpublished data). To examine the possible interaction of these two systems and confront the nullifying effect of the drugs on each other, the effective and the sub-effective doses of the pharmacological agents were administered in the co-injections. Thus, the sub-effective doses of L-arginine and L-NAME were administered in experiments 3, 4, 5 and 6 and the effective dose of L-arginine was injected in experiments 7 and 8.

Statistical analysis

Cumulative food intake was analyzed by repeated measures two-way analysis of variance (ANOVA) and the significant difference of food intake was detected by Tukey–Kramer test ($P < 0.05$). Results are presented as mean ± standard errors of the mean (SEM).

Results

The food intake response to ICV injection of L-arginine and L-NAME in neonatal layer-type chicks is illustrated in Figs. 1 and 2, respectively, and interaction of central nitrergic and GABAergic systems on food intake in neonatal layer-type chicks is shown in Figs. 3, 4, 5, 6, 7, and 8.

In experiment 1, the effect of ICV injection of different doses of L-arginine (200, 400 and 800 nmol), NO donor was examined on cumulative food intake in neonatal layer-type chicks and the effective and sub-effective doses of L-arginine were determined. In this experiment, ICV injection of 400 and 800 nmol of L-arginine decreased cumulative food intake in a dose-dependent manner at time-points 30, 60 and 120 min after injection ($P < 0.001$), but 200 nmol of L-arginine had no effect on cumulative food intake at time-points 30, 60 and 120 min after the injection

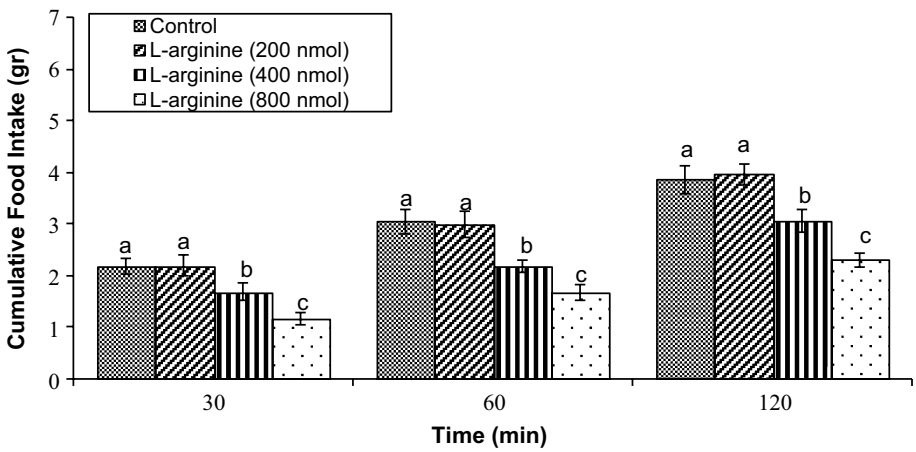

Fig. 1 Effect of ICV injection of L-arginine (a donor of NO) at different doses on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) (n = 8–11 per group)

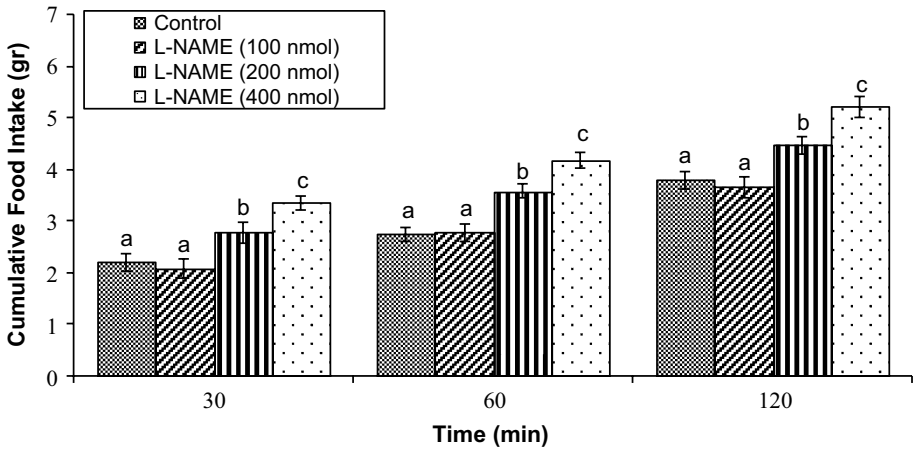

Fig. 2 Effect of ICV injection of L-NAME (a NO synthase inhibitor) at different doses on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) ($n = 9$–11 per group)

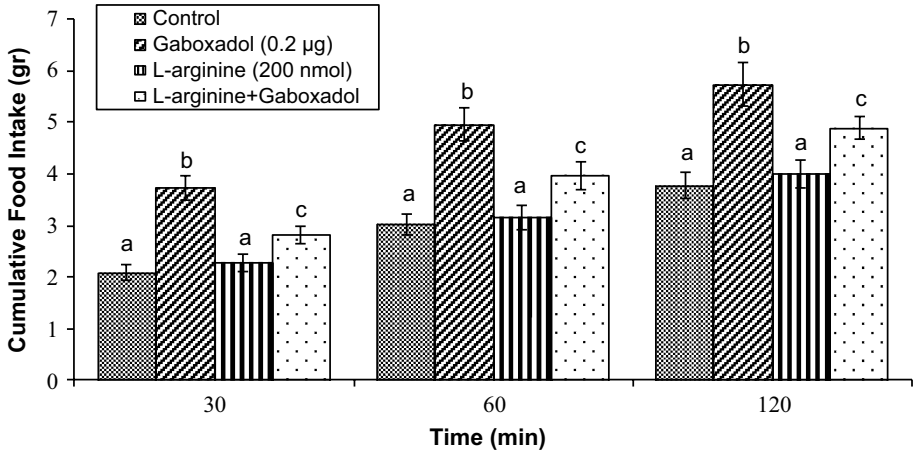

Fig. 3 Effect of ICV co-injection of Gaboxadol (0.2 µg) and L-arginine (200 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) ($n = 9$–11 per group)

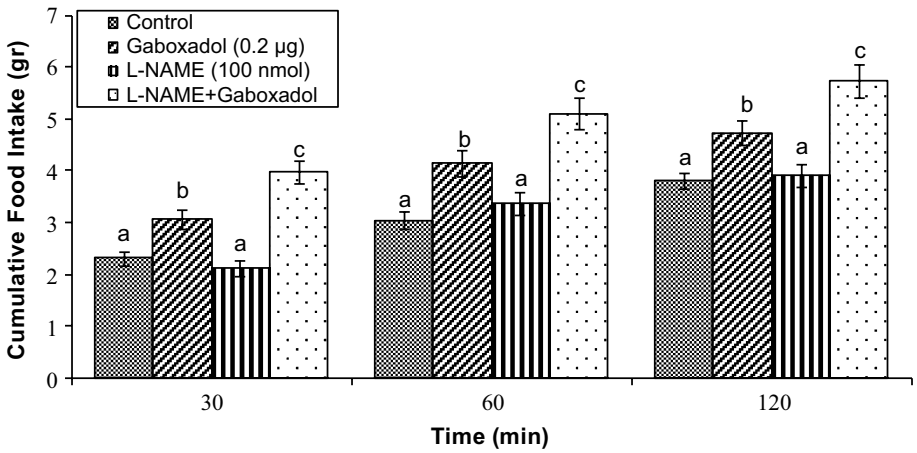

Fig. 4 Effect of ICV co-injection of Gaboxadol (0.2 µg) and L-NAME (100 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) ($n = 9$–11 per group)

($P > 0.05$) (Fig. 1). Therefore, 200 nmol of L-arginine was selected as a sub-effective dose in experiments 3 and 5 and 800 nmol of L-arginine was selected as an effective dose in experiments 7 and 8.

Experiment 2 was designed to examine the effect of ICV injection of different doses of L-NAME (100, 200 and 400 nmol), NO synthase inhibitor, on cumulative food intake in neonatal layer-type chicks. ICV injection of 200

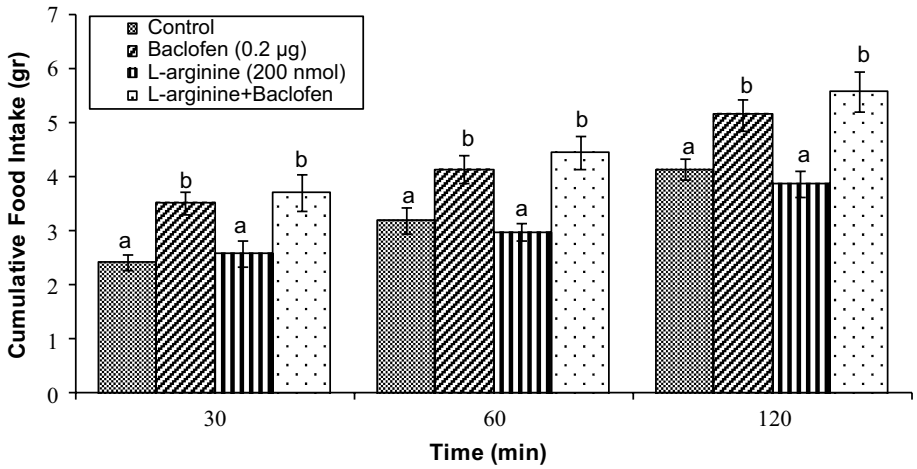

Fig. 5 Effect of ICV co-injection of Baclofen (0.2 µg) and L-arginine (200 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a* and *b*) indicate significant difference between the treatments ($P < 0.001$) ($n = 8$–11 per group)

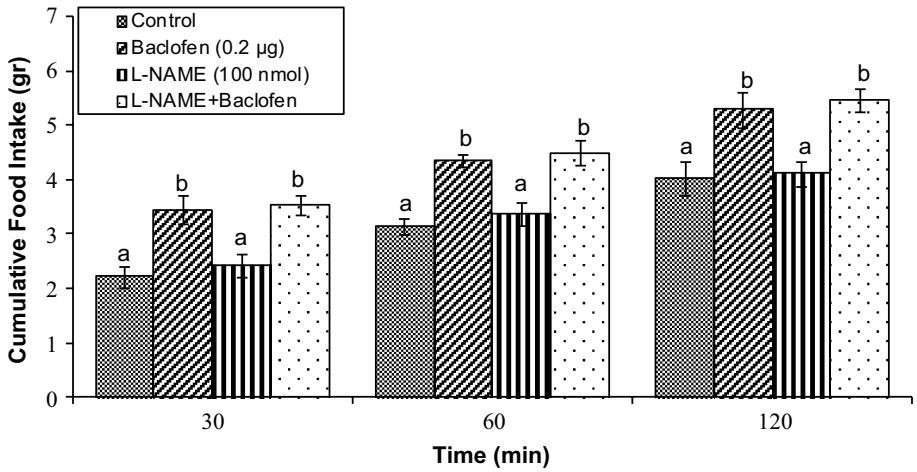

Fig. 6 Effect of ICV co-injection of Baclofen (0.2 µg) and L-NAME (100 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a* and *b*) indicate significant difference between the treatments ($P < 0.001$) ($n = 9$–11 per group)

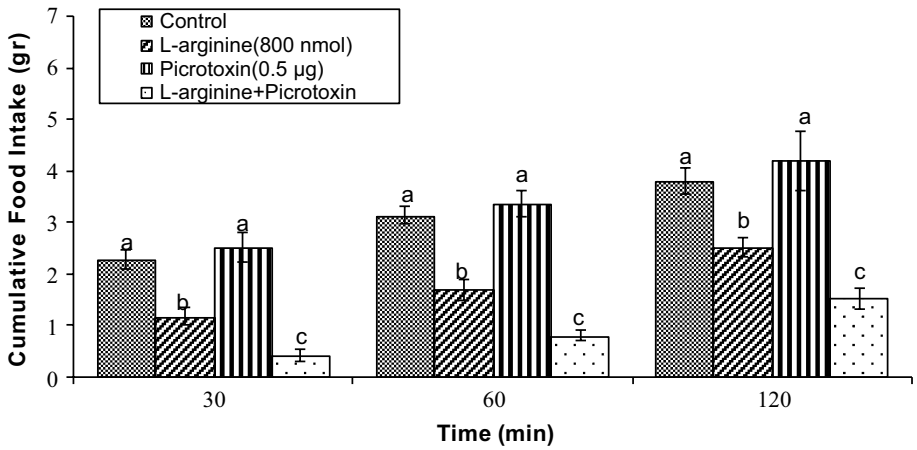

Fig. 7 Effect of ICV co-injection of Picrotoxin (0.5 µg) and L-arginine (800 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) ($n = 8$–11 per group)

and 400 nmol of L-NAME increased cumulative food intake in a dose dependent manner at time-points 30, 60 and 120 min after the injection ($P < 0.001$), but 100 nmol of L-NAME had no effect on cumulative food intake at time-points 30, 60 and 120 min after the injection ($P > 0.05$) (Fig. 2). Therefore, 100 nmol of L-NAME was used as a sub-effective dose for the experiments 4 and 6.

The results of experiments 1 and 2 showed the anorexigenic effect of central nitrergic system on food intake in neonatal layer-type chicks.

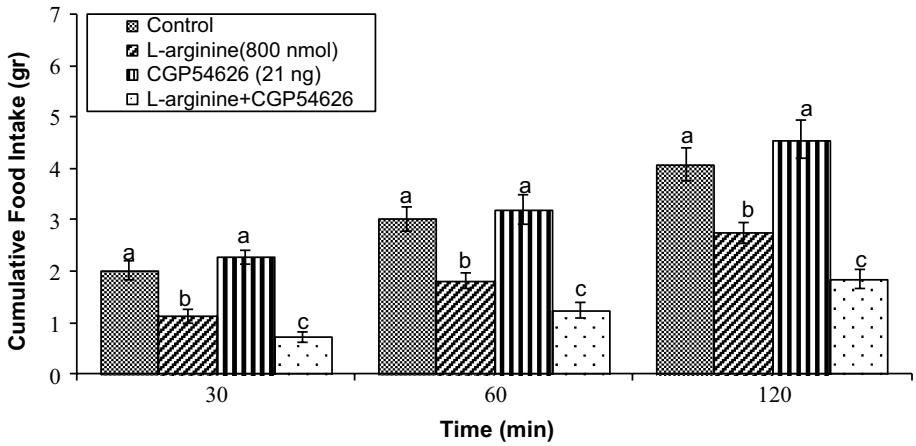

Fig. 8 Effect of ICV co-injection of CGP54626 (21 ng) and L-arginine (800 nmol) on cumulative food intake in neonatal layer type chicks. Data are expressed as mean ± SEM. *Different letters* (*a*, *b* and *c*) indicate significant difference between the treatments ($P < 0.001$) ($n = 9$–11 per group)

The results of experiment 3 showed that gaboxadol (0.2 µg), a $GABA_A$ agonist, significantly increased the amount of cumulative food intake at time-points 30, 60 and 120 min after the injection ($P < 0.001$), but the sub-effective dose of L-arginine (200 nmol) did not alter the cumulative food intake at time-points 30, 60 and 120 min after the injection ($P > 0.05$). In addition, the orexigenic effect of gaboxadol (0.2 µg) was significantly attenuated in the co-injection of L-arginine (200 nmol) and gaboxadol (0.2 µg) at time-points 30, 60 and 120 min after the injection ($P < 0.001$) (Fig. 3).

In experiment 4, injection of gaboxadol (0.2 µg) increased the amount of food intake at time-points 30, 60 and 120 min after the injection ($P < 0.001$), while the sub-effective dose of L-NAME (100 nmol) could not alter the food intake at time-points 30, 60 and 120 min after the injection ($P > 0.05$). In addition, the orexigenic effect of gaboxadol (0.2 µg) was significantly amplified in the co-injection of gaboxadol (0.2 µg) and L-NAME (100 nmol) at time-points 30, 60 and 120 min after injection ($P < 0.001$) (Fig. 4).

The results of experiments 3 and 4 showed that there is an interaction between GABAergic and nitrergic systems on food intake in neonatal layer-type chicks and orexigenic effect of $GABA_A$ receptor agonist is mediated via nitrergic system in CNS.

In experiment 5, baclofen (0.2 µg), a $GABA_B$ agonist, increased the amount of food intake at time-points 30, 60 and 120 min after injection ($P < 0.001$), but the sub-effective dose of L-arginine (200 nmol) did not alter food intake at time-points 30, 60 and 120 min after injection ($P > 0.05$). Furthermore, the orexigenic effect of baclofen (0.2 µg) was similar to the co-injection of L-arginine (200 nmol) and baclofen (0.2 µg) at time-points 30, 60 and 120 min after the injection ($P > 0.05$) (Fig. 5).

In experiment 6, baclofen (0.2 µg) significantly increased the amount of food intake at time-points 30, 60 and 120 min after injection ($P < 0.001$), while the sub-effective dose of L-NAME (100 nmol) had no effect on food intake at time-points 30, 60 and 120 min after injection ($P > 0.05$). Furthermore, the orexigenic effect of baclofen on food intake was similar to the co-injection of L-NAME (100 nmol) and baclofen (0.2 µg) ($P > 0.05$) (Fig. 6).

The results of experiments 5 and 6 showed that orexigenic effect of $GABA_B$ receptor agonist is not mediated via central nitrergic system in neonatal layer-type chicks.

In experiment 7, ICV injection of the effective dose of L-arginine (800 nmol) significantly decreased the amount of food intake at time-points 30, 60 and 120 min after the injection ($P < 0.001$), but picrotoxin (0.5 µg), a $GABA_A$ antagonist, had no effect on food intake at time-points 30, 60 and 120 min after injection ($P > 0.05$). Additionally, hypophagic effect of L-arginine (800 nmol) was significantly amplified in the co-injection of L-arginine (800 nmol) and picrotoxin (0.5 µg) ($P < 0.001$) (Fig. 7).

In experiment 8, L-arginine (800 nmol) significantly decreased food intake at time-points 30, 60 and 120 min after injection ($P < 0.001$), while the sub-effective dose of CGP54626 (21 ng), a $GABA_B$ antagonist, had no effect on the food intake at time-points 30, 60 and 120 min after injection ($P > 0.05$). Also, hypophagic effect of L-arginine (800 nmol) was significantly amplified in the co-injection of L-arginine (800 nmol) and CGP54626 (21 ng) at time-points 30, 60 and 120 min after the injection ($P < 0.001$) (Fig. 8).

The results of experiments 7 and 8 showed that anorexigenic effect of central nitrergic system on food intake in neonatal layer-type chicks was altered by $GABA_A$ and $GABA_B$ receptor antagonists (picrotoxin and CGP54626). On the other hand, anorexigenic effect of nitrergic system is mediated via $GABA_A$ and $GABA_B$ receptors in neonatal layer-type chicks.

In sum, these results probably suggest an interaction between central nitrergic and GABAergic systems on food intake in neonatal layer-type chicks.

Discussion

Our understanding of the cellular and molecular mechanisms that integrate food intake regulation with energy sensing in poultry remains quite limited. In fact, most of the discoveries concerning the regulation of appetite and energy expenditure have come from studies involving mammalian species. Because feeding and energy homeostasis are fundamental actions necessary for survival, it is logical to assume that the regulatory mechanisms governing these processes would be highly conserved in all animals (Kuenzel et al. 1999). However, it is also clear that there are distinct functional differences in food intake regulation between birds and mammals. Thus, the present study was designed to elucidate the probable interaction of central nitrergic and GABAergic systems on food intake in neonatal layer-type chick.

It has been shown that NO is a neuronal regulator of food intake in mammals (Morley et al. 2011) and birds (Choi et al. 1994). L-Arginine (a NO donor) is considered as an orexigenic factor (Morley and Flood 1991) and central administration of L-NAME (an inhibitor of NO synthase) decreased food intake in mammals (De Luca et al. 1995). However, the results of this study showed that ICV injection of L-arginine in a dose-dependent manner decreased food intake and L-NAME increased food intake in FD_3 neonatal layer-type chicks (Figs. 1, 2). Therefore, NO might act as an anorexigenic mediator in the brain of neonatal layer-type chicks and previous study showed that central administration of L-NAME stimulates food intake in layer-type chicks (Khan et al. 2007). Although, there are contradictory data in meat-type chicks in which central administration of L-NAME stimulates food intake (Khan et al. 2008), administration of L-NNA, an inhibitor of NO synthase, inhibited food intake (Choi et al. 1995). These controversial data might be the consequence of the injection methods, different strains and various food intake regulatory mechanisms between mammals and birds.

ICV injection of muscimol, a $GABA_A$ agonist, into the PVN of rat increased food intake (Stratford and Wirtshafter 2013). It is also reported that ICV injection of muscimol dose dependently increased food intake in turkey (Denbow 1991) and meat-type chicks (Jonaidi et al. 2002; Zendehdel et al. 2009). In this study, gaboxadol, a $GABA_A$ agonist, increased food intake in neonatal layer-type chicks (Figs. 3, 4) and previous study showed that ICV injection of muscimol induced hyperphagia in layer-type chicks (Bungo et al. 2003). These data and the results of this study showed that $GABA_A$ receptors have an orexigenic effect on food intake in mammals and birds.

One study reported that when a NO-active drug was co-administered with GABA, significant changes in GABA-induced responses were observed in subthalamic nucleus (STN) neurons; generally, decreased magnitudes of GABA-evoked responses were observed during continuous *S*-nitroso-glutathione (SNOG), an NO donor ejection, whereas the administration of L-NAME enhanced GABA responses (Sardo et al. 2009). These data might corroborate the reduction of anorexigenic effect of central nitrergic system on food intake in neonatal layer-type chicks by gaboxadol. Because, the orexigenic effect of gaboxadol (0.2 µg) was significantly attenuated in ICV co-injection of L-arginine (200 nmol) and gaboxadol (0.2 µg), but it was significantly amplified in ICV co-injection of L-NAME (100 nmol) and gaboxadol (0.2 µg).

Previous data showed the synergistic effect of GABA agonist and NOS inhibitor (Rawls et al. 2006) and in this study, the orexigenic effect of gaboxadol (0.2 µg) was significantly amplified in the co-injection of gaboxadol (0.2 µg) and L-NAME (100 nmol) even more than the group with ICV injection of gaboxadol (0.2 µg) alone, but the subeffective dose of L-NAME (100 nmol) did not alter the amount of the food intake in neonatal layer-type chicks (Fig. 4). These results probably indicate the synergistic effect of gaboxadol and L-NAME on food intake in neonatal layer-type chicks.

Previous study showed that picrotoxin, a $GABA_A$ receptor antagonist, had no effect on food in neonatal chicks (Takagi et al. 2003) and in this study, ICV injection of picrotoxin (0.5 µg) did not change the cumulative food intake in neonatal layer-type chicks (Fig. 7). In addition, the hypophagic dose of L-arginine (800 nmol) was amplified in the co-injection of picrotoxin (0.5 µg) and L-arginine (800 nmol) (Fig. 7). This result might be rationalized by a previous study which revealed that the NTS neurons have basal NO production and bicuculline, a $GABA_A$ receptor antagonist, increased basal production of NO in the NTS (Pajolla et al. 2009).

So far, based on the mentioned studies and the results of this study, there might be an interaction between the central nitrergic and GABAergic system via $GABA_A$ receptors on food intake in neonatal layer-type chicks. The mechanism of this interaction might be mediated by NO inhibitory synaptic inputs on GABA terminals via a cGMP-independent mechanism. As it was reported, NO preferentially potentiates the inhibitory synaptic inputs into supraoptic nucleus neurons by acting on GABA terminals in the supraoptic nucleus, possibly via a cGMP-independent mechanism (Ozaki et al. 2000) and in this study, the orexigenic effect of gaboxadol (0.2 µg) was reduced in the co-injection of gaboxadol (0.2 µg) and L-arginine (200 nmol) (Fig. 3).

Previous report showed that baclofen, a $GABA_B$ agonist, increased food intake in neonatal chicks (Bungo et al. 2003) and in this study, the amount of cumulative food intake in neonatal layer-type chicks was increased by baclofen (Figs. 5, 6). Nevertheless, in the co-injection

of baclofen (0.2 μg) with L-arginine (200 nmol) (Fig. 5) or L-NAME (100 nmol) (Fig. 6), the orexigenic effect of baclofen (0.2 μg) did not change. Therefore, orexigenic effect of baclofen was not altered by central nitrergic system in neonatal layer-type chicks. Also, baclofen was ineffective in reducing non-contact penile erection or yawning induced by drugs or physiological stimuli or the NO increase in the PVN of the hypothalamus (Melis and Argiolas 2002) and antinociceptive effect of baclofen was not modulated by nitrergic system (Przesmycki et al. 1999). Additionally, ICV injection of CGP54626 (21 ng), a $GABA_B$ antagonist, could not alter food intake in neonatal layer-type chicks (Fig. 8) and a former report showed that CGP54626 has no effect on food intake in neonatal chicks (Takagi et al. 2003). But, the hypophagic effect of L-arginine (800 nmol) was significantly amplified in the co-injection of CGP54626 (21 ng) and L-arginine (800 nmol) (Fig. 8). These data might show that $GABA_B$ receptors have a minor role in the interaction of nitrergic and GABAergic systems on food intake in neonatal layer-type chicks.

Conclusion

In summary, based on the results of this study, there might be an interaction between central nitrergic and GABAergic system on food intake in neonatal layer-type chicks and $GABA_A$ receptors play a major role in this interaction. Further investigations are required to elucidate the exact underlying cellular and molecular pathways of this interaction in neonatal layer-type chicks.

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Compliance with ethical standards

Conflict of interest Kasra Mokhtarpouriani, Morteza Zendehdel, Hossein Jonaidi, Vahab Babapour and Parviz Shayan declare that they have no conflict of interest.

Human and animal rights All experiments were executed in accordance with 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 the authors.

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