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The controlling role of nitric oxide within the shell of nucleus accumbens in the stress-induced metabolic disturbance

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ABSTRACT

Context and objectives: The involvement of the nitricergic system within the shell part of the nucleus accumbens (NAc) was evaluated in the metabolic disturbances due to stress.

Materials and methods: Male Wistar rats were cannulated in the shell of the left NAc. They received either saline or different doses of L-arginine and/or L-NAME five minutes before each stress session, for four days. Plasma cortisol concentration, food and water intake, time elapsing for eating, animal weight changes and adrenal gland weight were recorded.

Results: The L-arginine 1 μg/rat decreased the level of cortisol, water and food intake and time of feeding and increased the adrenal weight. But L-NAME at 1 μg/rat had opposite effects on these factors. However, the drugs showed similar effects at 10 μg/rat.

Conclusion: Injection of nitric oxide modifiers into the left side of NAc shell part may have an interactive role with sub-chronic stress in metabolic behaviour.

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Nitric oxide; L-arginine; L-NAME; stress; shell part of nucleus accumbens

Introduction

The most important part of the body affected by stress is brain. Previous studies have shown that, following stress, many neurological and hormonal changes occur in the organism. Studies show that wide range of changes occurred in the central nervous system after stressful event, which prepare the organism to overcome the stress side effects(Yaribeygi *et al.* 2017). The response to stress generated in the hypothalamus-pituitary-adrenal (HPA) axis that release glucocorticoid hormones such as cortisol hormone as well as sympato-adrenal axis (SA) that release epinephrine and norepinephrine (McEwen 2007). In addition, increasing of catecholamines (adrenaline and noradrenaline) and the secretion of neuropeptides such as vasopressin in the blood, which can causes some changes in the electrical properties, synaptic formation, and changes in the cell proliferation capacity in the brain (Yaribeygi *et al.* 2017). According to these data, one can conclude that stress has expanded effect on the several functions of brain such as memory, cognition, metabolic and motor control and motivation (Bahari *et al.* 2018, Mortazaei *et al.* 2018).

The nucleus accumbens, (NAc), as one of the most important structures of forebrain, plays an important role in the natural rewards, like eating and drinking behaviour, as well as the pharmaceutical rewards. According to the immuno-histochemical and morphometric studies, the nucleus is comprised from two distinct compartments namely the shell part and core. The shell part also serves as one of the extended amygdala consists which corporates as a link between reward and motivation. Cytoarchitector studies indicated that shell part has many nerve fibres and few cells (Lopez *et al.* 2008). Dopaminergic terminals reach NAc from ventral tegmental area (VTA), also glutaminergic terminals reach NAc shell part from other brain parts such as the prefrontal cortex, the hippocampus, thalamus and amygdala. There is also an orexinergic type of peptidergic relationship between the lateral hypothalamus and this part of the NAc (Sofiabadi *et al.* 2014). There is a small number of neurons in this part including small GABAergic interneurons (Afanas' ev *et al.* 2000). In addition, this part of the NAc is part of an operating system called the extended amygdala, which, along with the central nucleus of the amygdala and several other compartement, plays an important role in the instrumental conditioning and the emergence of the emotional part of addiction (Di Chiara 1999). Besides that, this part has a significant role in responding to stress (Kalivas and Duffy 1995). Previous studies have indicated that stress causes neural remodelling and enhances the activity of this part of the nervous system by the stimulation of the glutamate and GABAergic neuronal circuits in different parts of the amygdala, including the central nucleus (Singewald *et al.* 2000). Due to the important connections between the core of the amygdala and the shell part of the NAc, these effects are transmitted to this nucleus, and, on the other hand, any manipulation of the NAc shell part might affect the activities of the "expanded amygdala" (Groenewegen *et al.* 1999, Jackson and Moghaddam 2001). It is shown that, the activity

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of the glutamate system in the shell part of NAc mediated via it's ionotropic receptor, N-methyl-D-Aspartate (NMDA) receptors. One of the most important intermediates which is activated through the stimulation of NMDA receptors and is at least responsible for some effects of glutamate (Osanloo *et al.* 2015) is nitric oxide (NO). The NO is generated as the result of Ca^{+2} entry into the neurons because of the activity of NMDA glutamate receptors and the activation of the nitric oxide synthase in the neuronal cytoplasm (Wolf *et al.* 1994). Some investigators believed that, it is considered as a neurotransmitter which can affect both the pre and post-synaptic neurons (Hall 2015).

In the previous studies, the role of the NAc shell part as one of the extended amygdala segment has been studied in modulating the metabolic effects of stress in both male and female mices (Nicaeili *et al.* 2016) and rats (Javadifar *et al.* 2016, Ranjbaran *et al.* 2017). According to these studies, inhibition of NAc shell part decreases the effect of stress on metabolic behaviour. Besides that, in this regard, the role of glutamate system has been studied in both mice and rat(Lin and Pratt 2014, Osanloo *et al.* 2015). However, no research has been done on the role of nitric oxide in the NAc shell part in the incidence of these responses. On the other hand, the role of the nitric oxide in the NAc has been investigated in various behaviours such as conditioned place preference in male rats (Esmaeili *et al.* 2012) and, also, in the occurrence of morphine withdrawal syndrome (Sheibani *et al.* 2005). In this study, the effects of the activation and inactivation of the nitricergic system in the left part of NAc shell part was investigated on the incidence of metabolic disturbances induced by stress in male rats. It should be noted that, according to the previous studies, the left and right sides of the NAc shell part do not have the same function in modulating the stress performance. In fact, the left side of this nucleus has more pronounced effects on modulating the metabolic effects of stress than the right side (Nicaeili *et al.* 2016). The hypothesis of the current study was the fact that the modulation of the NO in the NAc, which is involved in the stress response, can modulate the effect of stress on many brain functions such as metabolic control.

Materials and methods

Animals

In this experimental and interventional study, 84 Wistar male rats weighing 120–180 g (purchased from the Pasteur Institute of Iran, Tehran) were used. The animals were housed in the cages of 6 (8 rats per cage), in 12:12-h light/dark cycles at 22–24° C temperature and provided with tap water and adequate rat chew (Pars Animal Feed Company-Iran). In each group of experiment, six animals were used. The amount of their water and food intake were recorded during the test. All experiments were carried out according to the laboratory animal use protocols of the Medical Ethics Committee of Baqiyatallah University of Medical Sciences.

Groups

The animals were randomly assigned into 14 groups. Those of the negative control group were cannulated in the left NAc. After that, saline was injected into the nucleus without inducing stress. In the animals of the positive control group, saline was again injected after the surgery for the cannulation of the left NAc. However, they expired electro foot shock stress for four days. The experimental groups were also laterally cannulated on the left side of the NAc. L-Arginine or L-NAME (1, 5 and 10 μg/rat) were injected for six groups followed by inducing stress after 5 min. For the other six groups, L-Arginine or L-NAME (1, 5, and 10 μg/rat) were also administrated without inducing stress.

Injection of NO modulators

The NO modulators used in this experiment were L-Arginine or L-NAME (Sigma-USA), which were injected into the nucleus. The animals were anaesthetised using diazepam hydrochloride (4 mg/kg) and ketamine hydrochloride (60 mg/kg). After the skull surgery, one stainless-steel guide cannula (23-gauge) was implanted into the animal's head using a stereotactic device, according to the coordinates of Atlas by Paxinos for the NAc (LA = 0.8, AP = 1.7, DV = 5.6 mm) (Sheibani *et al.* 2005). These cannula was placed 500 μ upper than the shell part of the NAc. To avoid the closure of cannula, a thin sterilised one stainless-steel wire was placed inside the guide cannula. After surgery and prior to the intra-NAc injections, the animals were left for 7 days in order to recovery from the surgery (Esmaeili *et al.* 2012). The injection cannula (30-gauge dental needle) which was conducted to a 1 μlit Hamilton syringe through a polyethylene tubing was placed into the guide cannula, while it's tip was 500 μm longer than the guide cannula. Then L-arginine or L-NAME was slowly injected (0.5 μlit/side) in the shell part of the left NAc in 60 s. At the end of the experiments, the animals were anaesthetised with high dose of ketamine and trans-cardiac perfusion with cool saline was performed. The brain were removed and histological studies were rapidly carried out to determine the location of the cannula.

Stress induction

Stress was induced using a communication box device (manufactured by the Tower Industry Corporation, Tehran, Iran). This device consists of 9 separate sections of 16 × 16 × 50 cm (length × width × height), made of plexy-glass. Its floor includes several stainless steel rods of 4 mm in diameter, spaced 1.30 centimetres from each other. These rods were connected to the generator, which is itself connected to the computer. Users (Osanloo *et al.* 2015) can determine the voltage and duration of the electrical current. In this study, electric foot shock (intensity: 40 mV, frequency: 10 Hz frequency) was induced randomly between 9:00 am to 16:00 pm for four consecutive days. The animals were brought to the test room one hour before testing and allowed them to adapt to the testing environment. After

that, they were placed in the device for 30 min, then, subjected to the electric foot shock for 60 s. Afterwards, the animals were kept in the device for additional 30 min. Notably, those animals of the negative control group and NO modulator groups without stress were kept in the device for 60 min without shock.

Blood sampling

On the first and last days of the metabolic recording, between 9:00 and 11:00 am, blood samples were obtained from the rats retro-orbital sinus, of all groups, the samples were centrifuged at 3000 rpm for 5 min. The collected plasma was used for cortical measurement using ELISA method (ELISA cortisol kit from DRG-Germany rat laboratory).

Measuring the amount of the water and food intake, and the time delay to start eating

At the end of each stress sessions, 10 g of rat chow and 100 cc of water were provided for each animal in the cage. On the next day, before the next stress session, NO modulator injection and inducing the shock, the amount of the consumed water and food during the day and night by minucing the amount of delivered from the amount of food/or water remained. This information had been recorded for each animal from the second day of the shock induction.

Every day, immediately after the shock, the animals were returned to their cages and an observer directly recorded the start time of eating. The start time of eating was recorded during the period of experiment, during 4 days.

Evaluation of the changes of the adrenal glands' weight

At the end of the experiment, the animals were anaesthetised by ketamine. Adrenal glands were removed surgically. These glands weighted using a sensitive digital scale.

Statistical analysis

The SPSS version 22 was used for statistical analysis. The results were expressed as the mean and plus/minus 95% confidence intervals. Three-way ANOVA was run to analyse the interaction effect of condition (stress, non-stress), NO modulators (L-arginine, L-NAME) and their doses (1, 5 and 10 μg/rat) on and adrenal gland weight and the plasma cortisol levels on the first and fourth day. The Mann–Whitney was used as a non-parametric test in order to compare the groups, as the number of samples in the groups was low. In order to analyse the metabolic changes during all days, the area under the curve (AUC) of the amount of weight, food and water intake and start time of eating were calculated. Then, three-way ANOVA followed by Bonferroni test was used for possible interactions: the condition, NO modulators and their doses on the AUC of the mentioned metabolic variables. $p < .05$ was considered as a statistically significant difference.

Results

Two effects of L-Arginine and L-NAME and stress on plasma cortisol in the first day

Three-way univariate ANOVA was used for comparing the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 μg/rat) and condition (stress, non-stress condition). There was significant interaction effect of NO modulator × dose × condition ($p < .02$). Since the Levene's test of homogeneity was significant ($p < .05$), the Mann–Whitney test was run for pairwise comparison. The results showed that there was no significant increase in the positive control group compared to the negative control. Injection of 1 and 10 μg/rat of L-Arginine and 10 μg/rat of L-NAME significantly decreased the plasma cortisol level in comparison with the control group, in both conditions, especially in the stress one ($p < .03$). The decreased effect of L-arginine at 1 μg/rat in the stress condition was even significantly more than the non-stress condition ($p < .03$). L-NAME at 1 μg/rat significantly increased the cortisol in the non-stress condition compared to the negative control group, and even the same dose in the stress condition ($p < .03$) (Figure 1). The comparison between the NO modulators showed that the level of cortisol after the administration of L-arginine at 1 μg/rat was significantly higher than the

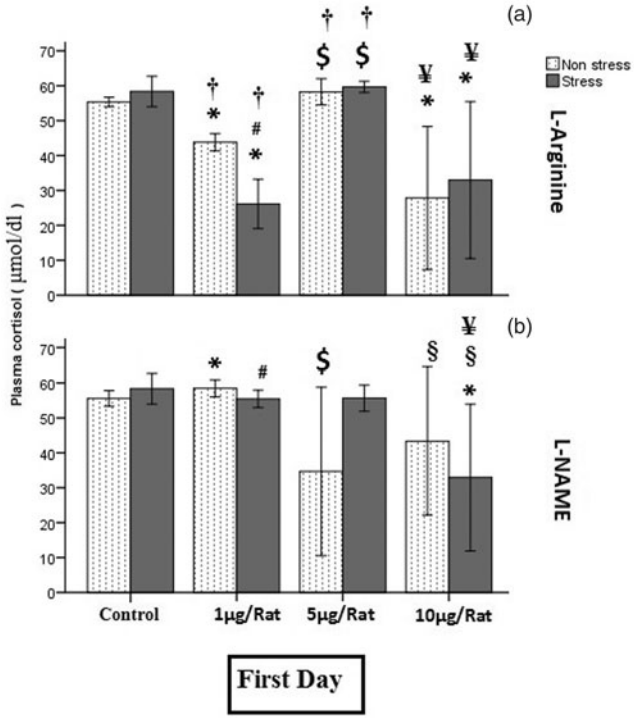

Figure 1. The mean (95% CI) of the cortisol level in the NO modulators (L-arginine, L-NAME), doses (1, 5, 10 μg/rat) and condition (stress, non-stress condition) on the first day. Plot (a) shows the level of cortisol in the L-arginine groups with or without stress and control groups. Plot (b) shows the level of cortisol in the L-NAME groups with or without stress and control groups. *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress conditions in each NO modulator and each dose. $: significance between 5 μg/rat and 1 μg/rat. §: significance between 1 μg/rat and 10 μg/rat. ¥: significance between 5 μg/rat and 10 μg/rat †: significance between two NO modulators in each condition and dose.

administration of the same dose of L-NAME, and, there was an inversed correlation at 5 μg/rat ($p < .03$) (Figure 1).

The effect of L-Arginine and L-NAME and stress on plasma cortisol on the fourth day

Three-way univariate ANOVA was run in order to analyse the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 μg/rat) and condition (stress, non-stress condition). There was significant interaction effect of NO modulator × dose × condition ($p < .009$). Since the Levene's test of homogeneity was significant ($p < .05$), the Mann–Whitney test was used for pairwise comparison. Indeed, stress, after four days, could increase the level of cortisol in the positive control group in comparison with the negative control group ($p < .03$). The injection of both NO modulators at 10 μg/rat decreased the level of cortisol compared to the control group in both conditions, and especially 1 μg/rat of the L-NAME ($p < .03$). L-arginine at 1 and 5 μg/rat decreased the level of cortisol, compared to the control group in the stress condition, but not in the non-stress condition ($p < .03$). L-NAME at 1 μg/rat increased the level of cortisol compared to the control group in both of the stress and non-stress conditions, and the same dose of L-arginine ($p < .03$) (Figure 2).

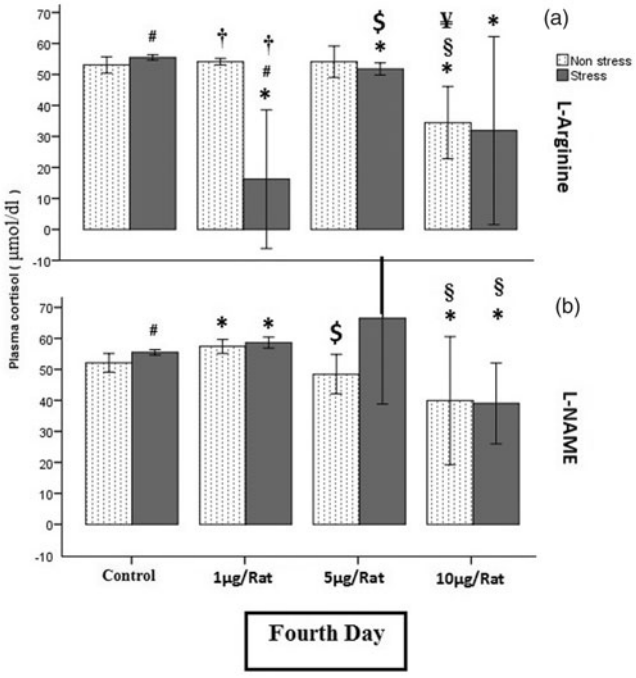

Figure 2. The mean (95% CI) of the cortisol level in the NO modulators (L-arginine, L-NAME), doses (1, 5, 10 μg/rat) and condition (stress, non-stress condition) on the fourth day. Plot (a) shows the level of cortisol in the L-arginine groups with or without stress and control groups. Plot (b) shows the level of cortisol in the L-NAME groups with or without stress and control groups. *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress condition in the NO modulator and each dose. $: significance between of 5 μg/rat and 1 μg/rat. §: significance between 1 μg/rat and 10 μg/rat. ¥: significance between 5 μg/rat and 10 μg/rat. †: significance between two NO modulators in each condition and dose.

The effect of L-Arginine and L-NAME and stress on the adrenal weight

Three-way univariate ANOVA test analysed the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 μg/rat) and condition (stress, non-stress condition). There was significant interaction effect of NO modulator × dose ($p < .000001$) and condition × dose ($p < .02$). Then, Bonferroni pairwise comparisons showed that the adrenal gland of rats received 1 μg/rat of L-arginine were heavier than that of the control group and the animals injected by other doses of L-arginine and all doses of L-NAME in each condition (with or without stress) ($p < .002$). On the other hand, adrenal gland of those rats injected by L-NAME at 5 μg/rat in the non-stress condition were lighter than the negative control group, 10 μg/rat ($p < .03$), the stress condition with the same does and other doses of L-arginine in both conditions ($p < .03$). The injection of L-NAME at 5 and 10 μg/rat, under stress, increased the adrenal weight in comparison with the positive control group ($p < .03$) (Figure 3).

The effect of L-Arginine and L-NAME and stress on the rat's weight during the 5-day evaluation

The change of the weight during the intervention is an important part of the metabolic evaluation. The sum of the area under the curve of weight during 5 days was calculated in each group. Here, 3-way univariate ANOVA was run to analyse the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 μg/rat) and condition (stress, non-stress condition). There was significant interaction effect of condition × dose ($p < .04$). The following Bonferroni pairwise comparisons showed that both of the NO modulators injected to shell part of NAc significantly decreased the rat's weight in the non-stress condition

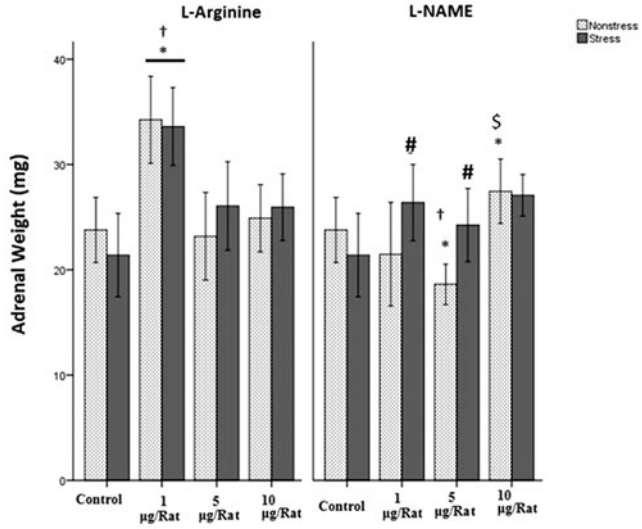

Figure 3. The mean (95% CI) of the adrenal weight in the NO modulators (L-arginine, L-NAME), doses (1, 5, 10 μg/rat) and conditions (stress, non-stress condition). *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress conditions in each NO modulator and each dose. $: significance between 10 μg/rat and 1 and 5 μg/rat. †: significance between two NO modulators in each condition and dose.

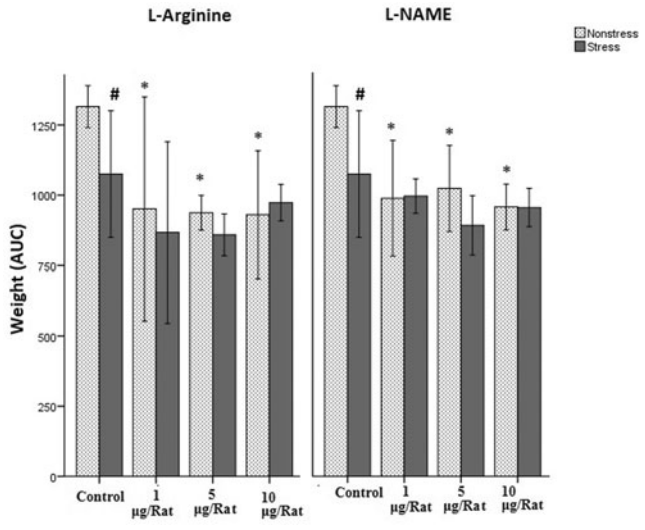

Figure 4. The mean (95% CI) of the sum of area under the curve of weight mean in five days of measurement. * Significance between doses of NO modulators and negative control group in the non-stress condition. #: Significance between the negative and positive control groups.

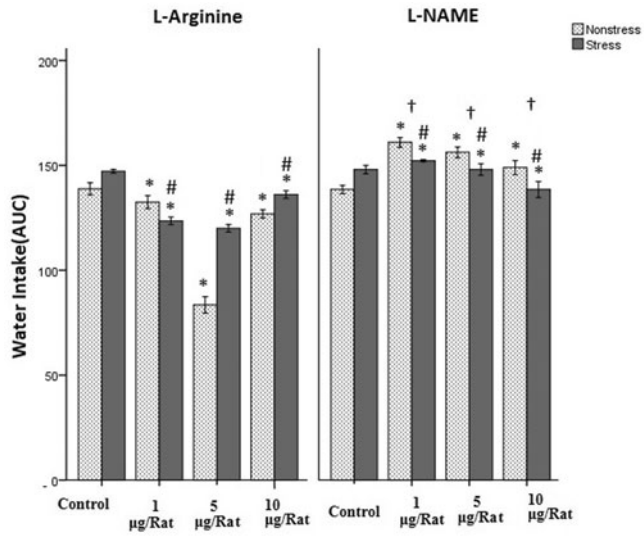

Figure 5. The mean (95% CI) of the sum of area under the curve of water intake mean in four days of evaluation. *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress conditions. †: significance between two NO modulators in each condition and dose. There were significance differences between all doses of each NO modulator that are not shown here. Pairwise comparison (Significance <.0001).

($p < .005$). No significant change was observed in the stress condition groups. The significant decrease was observed in the negative group in comparison with the positive groups ($p < .009$) (Figure 4).

The effect of L-Arginine and L-NAME administration and stress on the water intake during the 4-day evaluation

The amount of water intake during four days of intervention was calculated as the sum of the area under the curve of water intake for each group. Three-way ANOVA test analysed the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 µg/rat) and condition (stress, non-stress condition). There was a significant interaction effect of NO modulator × dose × condition ($p < .0000$). The Bonferroni pairwise comparison showed significant difference between all binary comparisons except for the positive control and L-NAME at 5 µg/rat group. In general, the significant differences showed that stress increased the water intake in the control rats. Injection of L-arginine and L-NAME significantly and inversely changed the amount of water intake. L-arginine decreased water intake especially at 5 µg/rat without stress. On the other hand, the injection of L-NAME increased the water intake in both conditions. However, the stress diminished the effect of L-NAME (Figure 5).

The L-Arginine and L-NAME and stress on food intake model during the 4-day evaluation

The amount of food intake during four days of evaluation and intervention, was calculated as the sum of the area under the curve of the food intake model mean for each group. 3-way univariate ANOVA test analysed the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 µg/rat) and condition (stress, non-stress condition). There was a significant interaction effect of NO modulator × dose × condition ($p < .02$). The Mann–Whitney test was used to compare the groups, as the Levene's test was significant ($p < .05$). The food intake model showed that only stress did not change food intake, but the NO modulators also changed it in different and dose-dependent patterns ($p < .009$). L-NAME at three doses and both conditions, except at 5 µg/rat in the stress condition, increased food intake and there was significant difference between doses in both conditions ($p < .009$). The increase of food intake occurred in the rats injected by L-arginine at 10 µg/rat in both conditions, and at 5 µg/rat in stress condition ($p < .009$). There was significant difference between doses in both conditions ($p < .009$). The injection of L-arginine and L-NAME at 1 and 5 µg/rat in stress condition significantly decreased food intake in comparison with non-stress condition ($p < .009$). Food intake of L-NAME groups were more than L-arginine groups in every dose ($p < .009$) (Figure 6).

The effect of L-Arginine and L-NAME administration and stress on the start time of eating during the 4-day evaluation

The start time of eating during four days of evaluation-intervention was calculated as the sum of the area under the curve of the start time of eating mean for each group. Three-way ANOVA test analysed the effect of three factors including NO modulator (L-arginine, L-NAME), dose (1, 5, 10 µg/rat) and condition (stress, non-stress condition). There was a significant interaction effect of NO modulator × dose × condition ($p < .003$). Bonferroni test was used to make comparison between the groups because the Levene's test was not significant ($p > .9$). The start of eating was significantly delayed following the stress ($p < .0000$), but both NO modulators had an opposite effect ($p < .009$) and decreased the effect of stress on the start time of eating

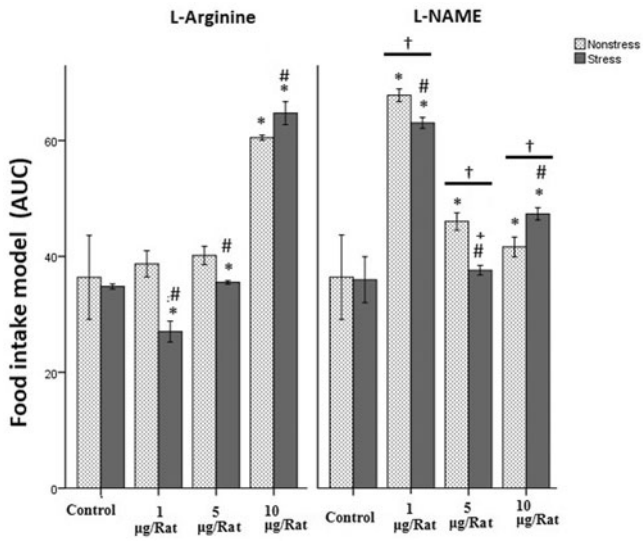

Figure 6. The mean (95% CI) of the sum of area under the curve of food intake mean in four days of evaluation. *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress conditions. †: significance between two NO modulators in each condition and dose. There were significance differences between all doses of each NO modulator that are not show here. Pairwise comparison (Significance <.009).

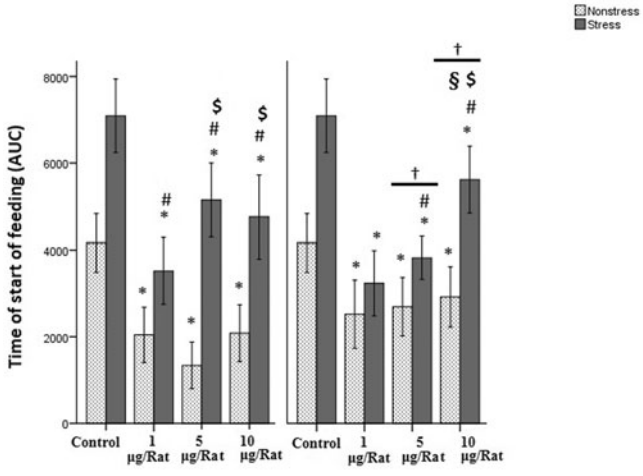

Figure 7. The mean (95% CI) of the sum of area under the curve of the start time of eating mean, during four days of evaluation. *: significance between each dose of NO modulator and their control in each condition. #: significance between stress and non-stress conditions. $: significance between 10 µg/rat and 1 µg/rat. §: significance between 10 µg/rat and 5 µg/rat. †: significance between two NO modulators in each condition and dose. Pairwise comparison (Significance <.01).

($p < .005$). There was significant delay in the time of eating with 10 µg/rat compared to other doses in the stress condition ($p < .01$) (Figure 7).

Discussion

In the present study, the effect of the nitricergic system, in the shell part of the left side of the NAc, has been investigated on the incidence of the metabolic effects of stress. The results verified the effectiveness of this system in the incidence of various metabolic effects, such as changes in the cortisol hormone, weight of the animals and adrenal gland and changes in the amount of water and food intake, and the start time of eating. Given the observed effects, it can be concluded that nitric oxide in the shell part of the left side of the NAc plays an important role in such behaviours that eventually control the individual's metabolism. Notably, this role may be exacerbated performed in the stress conditions.

The results of our study demonstrated that stress alone significantly increased the plasma cortisol level after four days without significant effect on adrenal weight. It is shown that repeated stimulation of the adrenocortical glandular cells through the released hormone from the pituitary gland during chronic stress increases the size and number of these cells (hyperplasia) (Bali and Jaggi 2015). Then we could conclude that the stress used in this study was sub-chronic. The administration of 1 and 10 µg/rat doses of L-arginine and 10 µg/rat doses of L-NAME reduced the plasma concentration of cortisol, especially under stress, and increased the adrenal glands' weight. However, 1 µg/rat decreased and 10 µg/rat significantly increased the amount of water and food intakes. But regarding L-NAME, 1 µg/rat and 10 µg/rat doses showed exactly the opposite effects on drinking and eating behaviour. In general, the effects of two NO modulators on delays in eating were different and even the opposite, especially on the first day. In addition, only 5 µg/rat of L-NAME reduced the weight of the adrenal gland in non-stress conditions. But all interventions, stress and NO modulators decreased the weight of rats.

However, it is now clear that stress changes the activity of several parts that modulate the metabolic behaviour (Mortazaei *et al.* 2018). According to the previous studies, the temporary inhibition of the NAc shell part in male rats could reduce the effects of stress on metabolic behaviour (Ranjbaran *et al.* 2017). Besides that, this temporary inhibition could also reduce the effects of acute stress in the female laboratory rats (Javadifar *et al.* 2016). In another study, the temporary inhibition of the NAc by lidocaine led to the inhibition of the metabolic effects of chronic stress in male laboratory mice (Nicaeili *et al.* 2016). All of these results indicate the role of the NAc, and especially its shell part, in mediating the effects of acute and chronic stress on metabolism.

The NAc shell part has side bias effect, and, therefore, there are some observed differences between the left and right sides of it. It should be noted that, according to the previous studies, the left side of the NAc shell part has revealed a better effect in terms of functionality in metabolism than the right side (Nicaeili *et al.* 2016). Because of that, this section was taken into account in the present study. In addition, previous research has demonstrated that glutamatergic inputs to the left NAc shell part are greater than the right side and may be one of the reasons for the functional differences between the two sides (Nicaeili *et al.* 2016). Interestingly, another study found that the inhibition of NMDA glutamate receptors in the left NAc by memantine led to the inhibition of the metabolic effects of stress in the small laboratory mice (Sarahian *et al.* 2015). It should be noted that since, due to the technical problems, there was

no possibility of the precise injection of memantine into the core of the NAc, the functional distinction between the shell part and central core of the NAc cannot be exactly investigated. Bear in mind that one of the ways of the glutamate effects exertion in various brain regions is the activation of NMDA receptors, which is associated with the stimulation of nitric oxide synthase (NOS) and increase in nitric oxide production (Moncada *et al.* 1991). Therefore, the effects of memantine may actually be due to inhibiting nitric oxide production.

Stress induces changes in gene expression of nNOS in regions related to stress responses and increases the production of NO (de Oliveira *et al.* 2000, Krukoff and Khalili 1997). Nitric Oxide increases the production of cyclic guanosine monophosphate (cGMP) by the activation of the guanylate cyclase. This secondary messenger is also responsible for a part of the nitric oxide's effects (Änggård 1994, Calabrese *et al.* 2007). NO is a free radical which is able to affect a wide range of bio-molecules in the membrane, cytoplasm and intercellular space, due to its radical nature, and makes them undergo nitrosylation. It also as a neurotransmitter is a multifunctional messenger that can transfer the signal in antero- and retrograde directions (Feil and Kleppisch 2008). Moreover, nitric oxide can interact with dopaminergic and glutaminergic systems in several brain areas such as NAc and increases the release of them (Motahari *et al.* 2016). On the other hand, the NAc is involved in the modulation of stress response (Ranjbaran *et al.* 2017). Therefore, NO modulation in the NAc can affect the stress-related response of brain such as metabolic control shown in the current study. In the present study, although stress increased cortisol plasma concentrations, decreased the rat's weight and changed the food and water intake, NO modulators affected these changes in different and dose-dependent manners.

Studies have demonstrated that the activation of the NAc activates the prefrontal cortex (Robinson and Kolb 1999), followed by the HPA axis inhibition (Gold 2015). Our results showed that 1 and 10 μg/rat doses of L-arginine and 10 μg/rat doses of L-NAME reduced cortisol levels in the stress and non-stress conditions. It is possible that the modulation of NO in the shell part of NAc has a retrograded effect (Feil and Kleppisch 2008) on more activation of prefrontal cortex's inhibitory role on hypothalamus and specially PVN.

The adrenal gland has two parts, medulla and core. The HPA axis terminates to the core, and sympathetic nerves stimulate the medulla part (Tsigos and Chrousos 2002). Previous studies have shown that, in chronic stress, the weight of the adrenal gland increased due to the increase in hyperplasia in the cerebrospinal region of the adrenal glands. This hyperplasia is due to the anterior pituitary hormone (ACTH) trophic effect on the activity of the adrenocortical fasiculatus cells, which can increase the volume and the number of these cells. Therefore, increase in the adrenal gland weight is one of the most important indicators of chronic stress (Bali and Jaggi 2015). Here, L-arginine at 1 μg/rat, that decreased the level of cortisol, increased the adrenal weight. It is proposed that VTA (Ventral tegmental area), which has a two-way communication with the NAc, is of two-way communications with locus coeruleus as the brain's noradrenaline source. The effects of locus coeruleus ultimately increase the modular activity of the adrenal gland (Mehendale *et al.* 2004, Haghparast *et al.* 2012, Ferrucci *et al.* 2013). Our results showed that all doses of L-arginine increased the weight of the gland in stress conditions, especially 1 μg/rat, with probably further stimulation of the brain stem and following the increase in the adrenergic system activity in the medullary of adrenal gland. However, we did not find any scientific discussion to confirm above hypothesis and more experiences are needed to clarify the phenomena.

In a study conducted by Sadeghi *et al.* (2016), stress did not affect the water intake, but reduced food intake and delayed the eating time. When the impact of stress on animal nutrition was evaluated, it was found that the feeding rate of animals did not change significantly, which contradicted with the reduction of post-stress nutrition in previous studies (Gluck 2006). Although, in a study by Sahraei et al., stress was found to increase the amount of food intake, and the latency of drinking, and did not have any significant effects on the amount of water intake, interestingly the amount of eating, the amount of drinking and the latency of the drinking decreased with the temporary suppression of the NAc (Gibson *et al.* 2010, Eftekhari *et al.* 2016). Paraventricular nucleus (PVN) is a main generator of several hormonal axes such as HPA. Notably, this nucleus has a dual role in the metabolic behaviour as well as the synergetic activity by the centres of satiety and hunger in the arcuate nucleus and the lateral hypothalamic nucleus respectively (Gibson *et al.* 2010). The NAc is an anterior brain region located in the anterior part of the hypothalamus. Therefore, it receives vague inputs from hypothalamus. Moreover, studies have shown that the NAc plays a role in the central autonomic regulation of nutrition (Mehendale *et al.* 2004), and this control is mainly done through the autonomic effects on the islets of Langerhans, hepatocytes and adipocytes (Steffens *et al.* 1990). Thus, the NAc can play a role in interfering gastro-intestinal signals associated with the proper digestive processing (Mehendale *et al.* 2004). The parasympathetic region of the hypothalamus also affects endocrine secretions due to its association with the PVN of the hypothalamus (Bernardis and Bellinger 1993). On the other hand, the cells of the NAc shell part indirectly inhibit the activity of the lateral cells of the hypothalamus (Castro *et al.* 2015). Thus, changing the activity of the NAc shell part would change the eating behaviour. The NAc shell part plays a role in controlling the swallowing behaviour because of the direct and indirect anatomical connections with the lateral hypothalamus. Previous studies have demonstrated that the stimulation of the NAc shell part increased biting in the animal (Robinson 1998). Based on the results, it seems that the relationship between the NO of NAc and the amount of drinking and eating is an inhibitory since the amount of food intake increased with L-NAME as a NO inhibitor and decreased with L-arginine as a NO activator. An interesting finding was that stress reduced the effect of L-arginine and L-NAME to change food and water intake except at 10 μg/

rat. Stress increased the delay in starting to eat that NO modulators decreased it.

In many studies, changes in plasma concentrations of corticosterone have been used as the main criterion of the HPA axis activation in rodents (Nicaeili *et al.* 2016). According to our result, firstly, cortisol hormone was detectable in the plasma of rats and, secondly, the plasma concentration of this hormone was different in the stress and non-stress conditions, indicating that this hormone is sensitive to stress. Therefore, we suggest that changes in plasma concentrations of this hormone can also be used to investigate the effects of stress on the activity of the HPA system in rodents.

It should be noted that the limitations of the current study were the low number of rats in each group and no measurement of NOS gene expression in the NAc region.

Conclusion

The results of our study demonstrated that stress alone significantly increased the plasma cortisol level after four days without significant effect on adrenal weight. The repeated stimulation of the adrenocortical glandular cells through the released hormone from the pituitary gland during stress increases the size and number of these cells (hyperplasia) (Bali and Jaggi 2015). Then we could conclude that the stress used in this study was acute effect. L-arginine at 1 and 10 μg/rat and L-NAME at 10 μg/rat significantly reduced the plasma cortisol and increased the adrenal weight, especially 1 μg/rat of L-arginine in both conditions. L-NAME and L-arginine showed reversal and dose-dependent effects on water intake but they had similar increasing effects on food intake. However, 1 μg/rat of L-arginine and L-NAME had opposite effects on the cortisol level of plasma and metabolic behaviour. Stress decreased their effect on food intake it except at dose of 10 μg/rat. The weight of the rat significantly decreased with both interventions. Stress increased the delay in staring to eat that was reduced after NO modulators' injection to NAc shell part. Thus, according to the results, the manipulation of the nitricergic system may affect its connections with the regions involved in the stress-inducing system and the metabolic regulation of their output, by the modification of inputs to NAc. Ultimately, the concentration of these materials has a direct and sometimes inverse effect. It is recommended to study some doses less than 1 μg/rat in the future.

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