Physiology and Pharmacology

Physiol Pharmacol 20 (2016) 220-230 www.phypha.ir/ppj

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Original Article

Transient inactivation of the central amygdala modulates metabolic and hormonal responses to acute stress in female rats

Tahereh-Sadat Javadifar[1], Hedayat Sahraei[2], Mohammad-Ali Ketabi[3], Mohammad Nasehi[1,4], Mohammad-Reza Zarrindast[1,4,5,6,7,8]*

1. Institute for Cognitive Science Studies (ICSS), Tehran, Iran
2. Neuroscience Research Center, Baqiyatallah (a.s) University of Medical Sciences, Tehran, Iran
3. Department of Endodontics, School of dentistry, AJA University of Medical Sciences, Tehran, Iran
4. Cognitive and Neuroscience Research Center (CNRC), Medical Genomic Research Center and Scholl of Advanced Siences in Medicine, Islamic Azad University, Tehran Medical Science Branch, Tehran,Iran
5. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
6. School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
7. Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran
8. Substance Abuse and Dependence Research Center, University of School Welfare and Rehabilitation Sciences, Tehran Iran

Abstract

Introduction: Current study examined the possible role of the central nucleus of amygdala (CeA) transient inactivation on the metabolic and hormonal disturbances induced by acute electro foot shock stress in female rats. Considering the differences between female and male in responses to stress, this study attempts to reveal possible mechanisms underlying these differences.

Methods: Uni- or bilateral CeA nucleus cannulation of female Wistar rats (W: 200±20 g) was preformed seven days before stress induction. Lidocaine hydrochloride (2%) administered five minutes before electro foot shock. Food and water intake, time of delaying the onset of eating, plasma glucose, corticosterone, estradiol and progesterone were measured after stress termination.

Results: Stress caused an increase in food intake and time of delaying the onset of eating whereas had no effect on the water intake. In addition, plasma glucose, corticosterone and progesterone concentrations were increased. The CeA inactivation in the right and left sides results in reduced water intake and increased delay times to eating. However, bilaterally inactivation of the CeA results in reducing time that elapsed before eating. Lidocaine administration in the both sides of nucleus had no effect on food intake. Transient inactivation of the bilateral sides of CeA augmented the stress effect on the plasma glucose and estradiol but had no significant effect on the corticosterone and progesterone hormones.

Conclusion: It could be concluded that inhibition of the CeA by lidocaine modulate certain metabolic and hormonal responses to acute stress in female rats. The CeA influence seems to be asymmetrical.

Keywords:
Acute stress;
Central Amygdala;
Corticosterone;
Estradiol;
Female rat;
Progesterone

Received: 19 Jul 2016
Accepted: 14 Sep 2016

***Correspondence to:**
M.R. Zarrindast

Tel: +98-2166402569
Fax: +98-2166402569

Email:
zarinmr@ams.ac.ir

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Introduction

Organisms reply to the environmental challenges and perceived threat with a coordinated set of psychophysiological reactions that are known as adaptive responses to stress (Pacak and Palkovits, 2001). Acute stress is a state of single, intermittent and time limited exposure to stressors (Koolhaas et al., 1997), which is contaminant by various physiological changes that include activation of the hypothalamic-pituitary-adrenal (HPA) axis that characterized by the release of adrenal steroids (cortisol in human and corticosterone in rats) triggered by the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH release is controlled, in turn, by the liberation of hypothalamic corticotropin-releasing factor (CRF) (Burchfield, 1979). Acute stress also induce activation of the other stress system namely sympathoadrenal system in which releases adrenaline and noradrenaline in response to stressors (Krizanova et al., 2016).

The amygdaloidal complex, located in the medial temporal lobe, is structurally divided into three groups. The first group is the deep or basolateral group, the second one is superficial or cortical-like group and the third group called centromedial that composed of the medial and central nuclei. According to numerous studies, central nucleus of amygdala (CeA) as an important part of extended amygdala plays an important role in the organization of stress responses (Charmandari et al., 2005) and numerous lesion studies have shown involvement of this nucleus in autonomic responses to stress (Cardinal et al., 2002; Williams et al., 2007). Previous studies have shown that electrical stimulation of the CeA increased heart rate, blood pressure, sympathetic outflow, respiratory rate and HPA axis activity (Merlo Pich et al., 1995). Since a rich plexus of CRF-containing terminals and neurons projecting to the brainstem is present in the CeA, it has been suggested that CRF in the CeA mediate the emotional responses to stress exposure (Merlo Pich et al., 1995). Therefore, CeA nucleus is a structure that plays a role in orchestrating various aspects of the emotional output. Moreover, the involvement of this nucleus in the regulation of the ingestive behaviors such as food intake, water intake and body weight have investigated (Ganaraj and Jeganathan, 1998; Valles et al., 2000). Other findings have suggested that the amygdala has important role in linking aversive stimuli to the neuroendocrine responses to psychological stress (Goldstein et al., 1996).

On the other hand, it is revealed that there are sex differences in certain aspects of stress responses. For example, psychiatric disorders in relation with stress have reported in more prevalent for females (Kendler et al., 1995). Numerous studies have shown that acute stress produced higher and more persistently elevated plasma corticosterone and ACTH concentrations in female rats compare with males (Kitay, 1961; Viau et al., 2005; Iwasaki-Sekino et al., 2009). Moreover, Valentino et al., in 2013 have shown that sex difference determine the direction of the receptor signaling for the CRF1 receptor signaling pathway that would render females more responsive to acute stress and less able to adapt to chronic stress.

Considering the importance of the CeA in the integration of responses to emotional events such as stress, there is little evidence about the role of this component of the extended amygdala in response to acute stress in female rats. Current study were designed to determine if transient inactivation of the CeA uni- or bilaterally induce any change in metabolic and neuroendocrine components of the stress response in female rats and determine whether these responses are coordinated concurrently within the CeA in females.

Materials and methods

Animals

All procedures in present study were performed in accordance with the guidelines established by the animal care and use committee of the Neuroscience Research Center, Baqiyatallah (a.s) University of Medical Sciences, Tehran, Iran. Adult female rats (W: 200±20 g) of Wistar strain (Pasture Institute, Tehran, Iran) were used in this study. The animals were housed four per cage (29cm x 22cm x 14cm) during the experimental period. The rats were maintained under standard laboratory conditions with 12h light: 12h dark cycle at 22±2 °C temperature and 50±5% humidity. Standard feed (standard pellets, Pars production and distribution of animal feed Company, Iran) and tap water in drinking bottles were made available ad libitum. Animals were kept in their home

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cages for one week before the beginning of experiments and their average consumption of food and water intake was measured as the base for food and water intake before stress induction. The animal place was equipped with the camera system to following the animals feeding and water intake.

Drugs

The drugs that were used in this experiment include lidocaine hydrochloride (Sigma, St. Louis, MO, USA), ketamine hydrochloride (50 mg/kg, ip) (Alfasan, The Netherland) and diazepam (5 mg/kg, ip) (Darupakhsh, Iran). Lidocaine was prepared in sterile saline and then injected into central amygdala in a volume of 0.25 µl/side 5 min before the stress induction (Moaddab et al., 2009; Esmaeili et al., 2012). The sham group received saline 5 min before stress induction. Ketamine and diazepam were injected to the animals in a volume of 1 ml/kg before each surgery.

Stress induction

The communication box (16×16×50 cm, length × width ×height) that was used for footshock induction by electrical current (1 mA current and 10 HZ frequency), was made from Plexiglas and consists of nine distinct compartments. This nine-chambered apparatus was designed to make the same condition for electrical stimulation for all of the rats that belonged to one group. The floor of the compartments equipped with the stainless steel rods (4 mm in diameter) that were connected to the electrical stimulation apparatus (Borj-e-Sanat Corporation, Tehran, Iran) which was controlled by the PC to generating an electric foot shock randomly 60 s during 100 s (Nasihatkon et al., 2014). This means that the electrical stimulation that each rat received during 100 s was in a random manner and in a total time of 60 s. This random stress induction in the certain duration was planned to avoid adaptation of rats to uninterrupted electric foot shock and this type of physical stress in all the times of induction became a new challenge for them.

Identification of estrous cycle

The estrous cycle phase of female rats was tested for five days (from four days before the stress day) between 8:00 and 9:00 am For this purpose animal's vaginal smears were taken and the estrous cycle

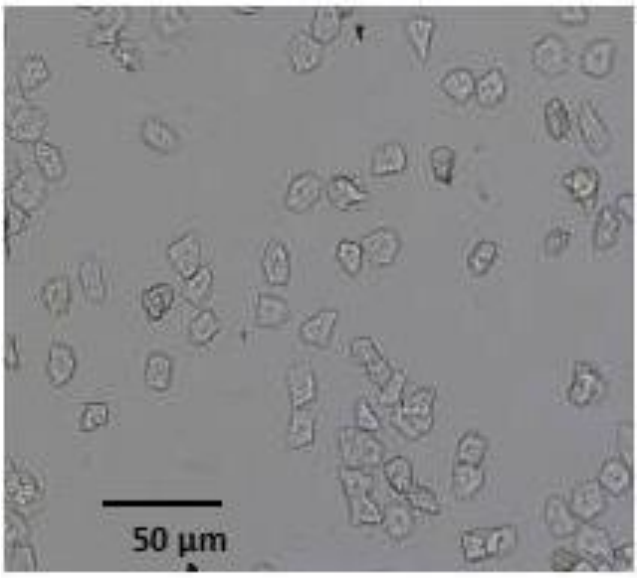

Fig.1. Evaluation of the estrous cycle phases. Proestrus phase was identified by predominant nucleated epithelial cells. Scale bars: 50µm

phases of them were detected by using light microscopic observation and morphologically evaluation of the cells. For the unification of the estrous cycle phase in all the rats that were involved in our experiments only the animals in the proestrous phase of the cycle were chosen (Fig. 1). This phase is known by the predominance of the nucleated epithelial cells in smear samples and the ovulation occurs from the beginning of this phase to the end of estrus phase (Marcondes et al., 2002). The reason that we have chosen the proestrus phase was that plasma level of estradiol in this phase is in its highest one (Marcondes et al., 2002) and also this is true for plasma corticosterone, which indicated that the HPA axis sensitivity is in its high level (Handa et al., 1994).

Experimental design

For avoidance from environmental adaptation in the animals, all the procedures of experiments were performed between 9:00 and 16:00 and also the cages of animals were carried to the experimental room 1 h before the beginning of the experiments. The animals that belong to stress groups (n=7 for right side group, n=7 for left side group and n=8 for bilateral group that all of them received lidocaine). Animals that belong to sham group (n=7) were received normal saline in the same volume of lidocaine with stress induction. These animals were placed in the communication box (one rat per each compartment) 30 min before electro foot shock

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induction and then after stress termination (100 sec), animals were left idem for an additional 30 min. The control group (n=8) rats were transported to the stress box for 60 minutes without receiving any drug or stress. Food and water intake by each rat were evaluated by measuring the percentage of food and water intake of 24 h after stress relative to the amount that was consumed by same rats 24 h before the stress induction. The time lag between the returning of rats to their home cages and restart to consumption of food was recorded as an index of stress severity. Blood sampling was performed immediately after removing the animals from the communication box (with or without stress).

Blood sampling

Glucose, corticosterone, estradiol and progesterone concentrations in both the control and stressed groups were measured from plasma samples that obtained from the blood of retro-orbital sinus. The blood samples for glucose measurements were taken immediately after stress termination. So, the animals were fasted for one hour (stress duration) each. For separating the plasma, 1 ml of blood combined with 0.1 ml of 3% EDTA solution and then samples were centrifuged at 3000 g for 7 min at 4 °C, the isolated plasma was kept at -20 °C until to use (Toleikis and Godin, 1995). Plasma glucose concentration was measured using the glucose oxidase method (Pars Azmoon, Iran). In present study hormonal concentrations were assessed using enzyme-linked immunosorbent assay (ELISA) kits for corticosterone (Rat Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany) and for estradiol and progesterone assessment (Diagnostics Biochem Canada Inc.). All of the hormone concentrations were read at 450 nm.

Surgical procedure

Ketamine hydrochloride (50 mg/kg, ip) and diazepam (5 mg/kg, ip) were used contemporary for anesthetizing the rats before surgery and cannulae implantation. The animals were placed in the stereotaxic apparatus in the position that the incisor bar set at 3.3 mm below horizontal zero to achieve a flat skull position. After exposing the rat skull via an incision, one or two guide cannulae (23 gauges) were placed stereotaxically (Stolting Instruments, USA) into the central nucleus of the amygdala for animals

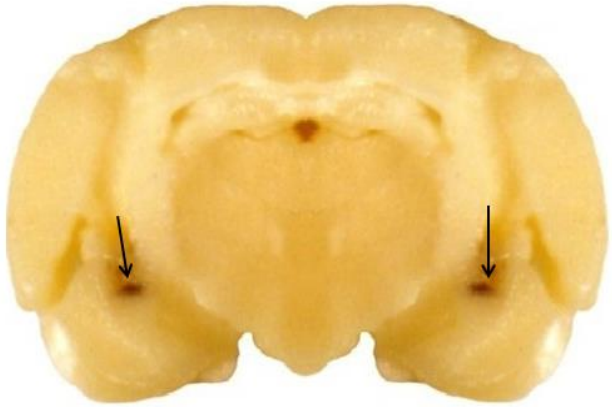

Fig.2. Histological verification of injection site in the CeA. Arrows showed the site of injection.

that receiving uni- or bilateral injections respectively, 0.5 mm above the intended site of injection according to Paxinos and Watson (2007) at stereotaxic coordinates: AP -2.52 mm posterior to bregma, and L ± 4.4 mm lateral to the sagittal suture and 7.8 mm below the top of the skull. The guide cannulae were secured with jewellers' screws and dental acrylic cement was used to closing the incision. Rats were allowed 5-7 days for recovering from the surgery and anesthesia. For drug infusion, the animals were gently restrained by hand and lidocaine solution was manually injected into the nucleus by using the 30-gauge injection needles that were designed 0.5 mm longer than guide cannula and connected by polyethylene tubing to a 5.0-µl glass Hamilton syringe. Total lidocaine volume in 0.25 µl /side over a 30-s period was injected to the considered nucleus. Injection needles were left in cannulae for an additional 60 s to facilitate diffusion of the drugs.

Histology

After the experiments were completed, all of the rats were deeply anaesthetized by diethyl ether and then received ink into the injection site (0.1 µl of 1% aquatic methylene blue solution) was injected into the guide cannulae to aid the histological verification. Transcardiac perfusion of 0.9% normal saline followed by 4% buffered formalin was performed before removing the brain. The removed brains were blocked and sections were taken through the brain areas of cannulae placements, and then the accuracy of the cannulae placements were verified (Fig. 2). According to the atlas of Paxinos and Watson, the tissues that their injection sites located outside of the

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nucleus were excluded from the analyses. The brains were evaluated using the light dissection microscopy.

Data analysis

All data are expressed as mean±SEM. Different responses to acute stress (in comparison with control group) and the inactivation of the CeA in each sides (in comparison with stress-saline group) was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Differences with $P<0.05$ were considered significant.

Results

Plasma corticosterone concentration changes after stress induction and inactivation of the central amygdala

Acute stress induced elevation of the plasma corticosterone concentration in female rats significantly compared with non-stress group [F (4, 29) = 17.27, $P \leq 0.001$]. The effects of stress on plasma corticosterone levels and the effectiveness of the inhibition of the right, left and both sides of CeA are shown in Fig. 3A. Results revealed that transient inactivation of the right, left and both sides of the CeA in the female rat resulted in no significant change in the corticosterone concentration in the comparison with sham group.

Evaluation of transient inactivation of the central amygdala on fluctuations of estradiol and progesterone hormones induced by acute stress

Progesterone concentration in plasma samples of sham group which received only acute stress increased in comparison with the non-stressed group [F (4, 30) = 10.34, $P \leq 0.001$]. In addition, transient inactivation of the central amygdala in right, left and bilateral sides caused to no significant change in the plasma progesterone concentration compared with sham group (Fig. 3B).

Acute stress in female rats didn't show any significant effect on the plasma estradiol concentration. In addition, inactivation of the central amygdala in the right and left sides had no significant effect on the estradiol concentration in comparison with non-stress and sham groups but bilateral inactivation of the CeA resulted in the significant increased estradiol concentration compared with sham group [F (4, 30) = 3.82, $P \leq 0.05$] (Fig. 3C).

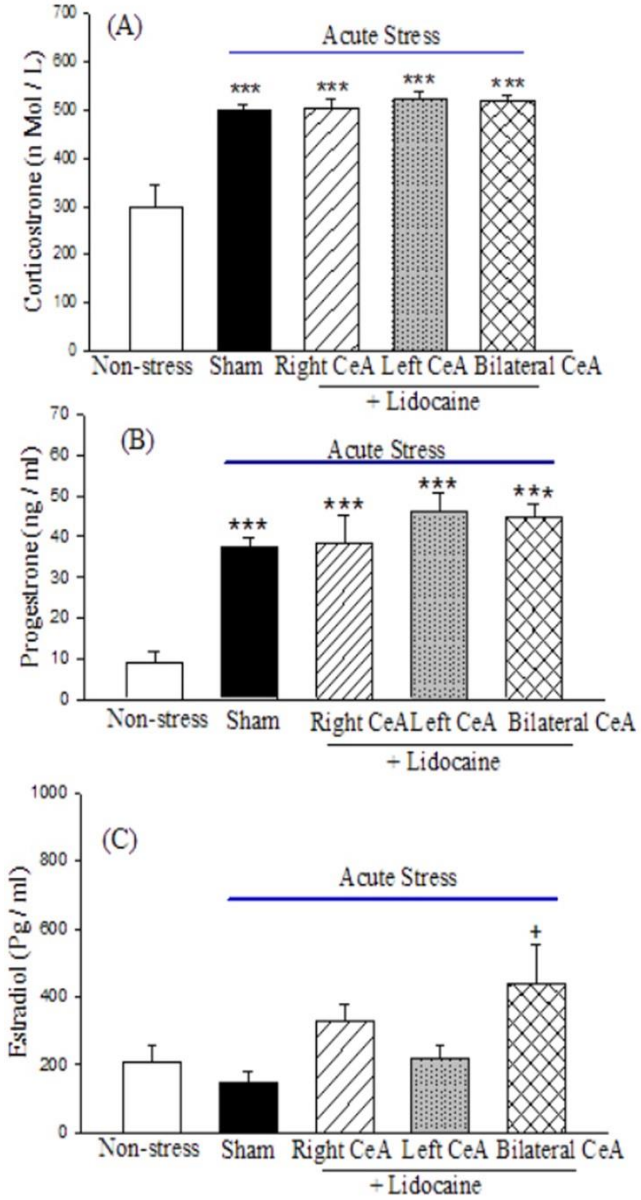

Fig.3. Effects of left, right and bilateral transient inhibition of the central nucleus of the amygdala on corticosterone (A), progestrone (B) and estradiol (C) plasma concentrations immediately after the induction of stress. Each point shows the mean±SEM of hormone concentration for 6-8 rats. ***P <0.001, different from the control group. ^{+}P < 0.05, different from the sham group.

Evaluation of the changes in feed and water intake and latency to eating after stress and inactivation of the central amygdala

According to our results acute stress in female rats caused to increased food intake [F (4, 34) = 11.28, $P \leq 0.001$]. In addition, lidocaine injection to the central amygdala uni- or bilaterally indicated no significant effect on food intake in comparison with sham group (Fig. 4A).

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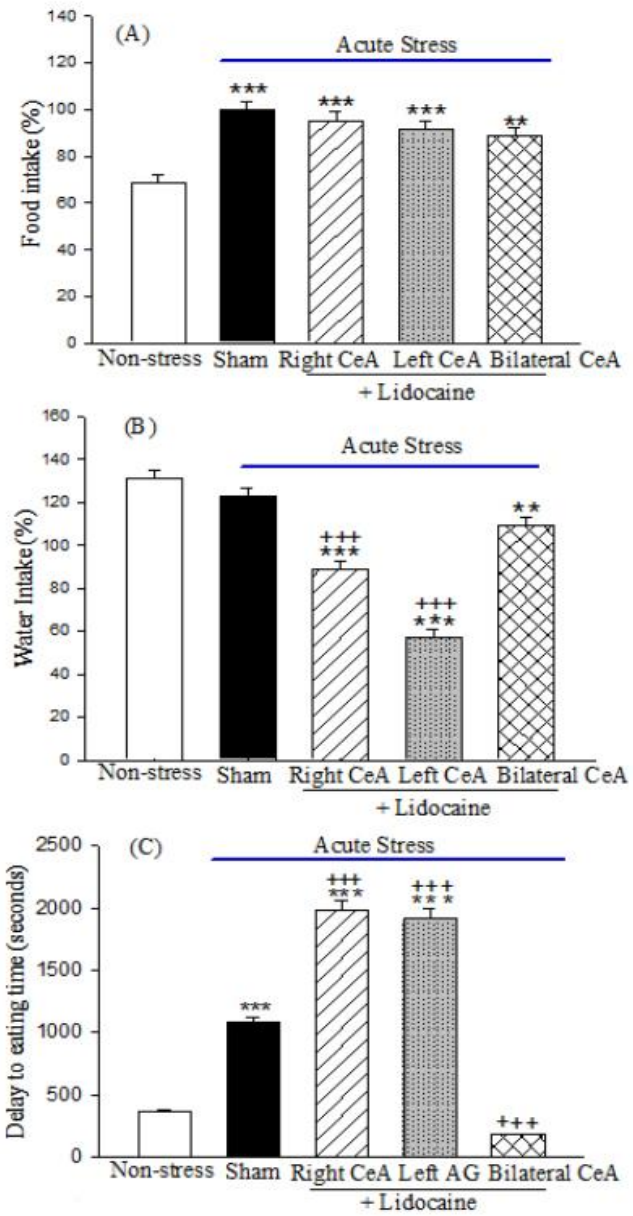

Fig.4. Effects of left, right and bilateral transient inhibition of the central nucleus of the amygdala on food intake (A), water intake (B) and delay to eating time (C) after acute stress induction. Each point shows the mean±SEM of food or water intake 24 h after stress induction (A, B) or time elapsed until animals began eating after returning to their home cage for 6-8 rats. ***P < 0.001, **P < 0.01 different from the non- stress group; ^{+++}P < 0.001, different from the sham group.

Results of water intake that was measured after acute stress for each animal indicated that stress alone had no significant effect on the water consumption. Furthermore, inhibition of the right and left sides of the central amygdala separately by lidocaine induced reduction in the water intake compared to non-stress and also sham groups significantly [F (4, 34) = 68.49, $P \leq 0.001$], whereas,

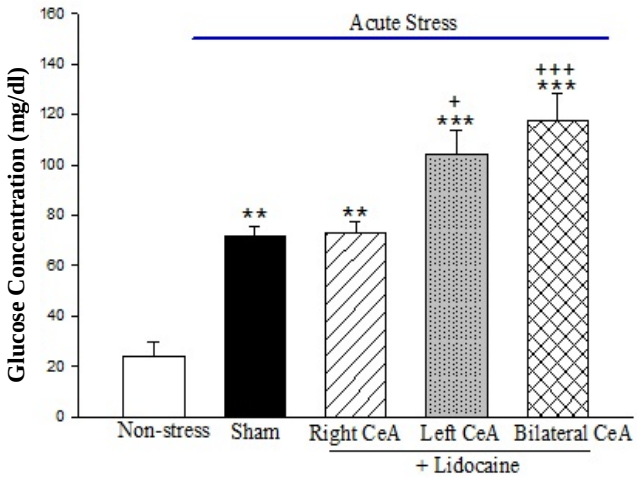

Fig.5. Fluctuation of glucose concentration in female rats that received acute stress with inactivation of right, left and bilateral sides of the central nucleus of the amygdala. Each point shows the mean±SEM of glucose concentration for 6 - 8 rats. ***P < 0.001, **P < 0.01 different from the non- stress group. ^{++}P < 0.01, ^{+}P < 0.05 different from the sham group.

bilateral inactivation of this nucleus leaded to reversing this effect and significant difference with sham group that was seen in unilateral inhibition is abolished (Fig. 4B).

Delay time to onset of eating represented the time that was elapsed by rats to beginning the eating after induction of the acute stress and returning back to their home cage. Results indicated that stress increased delay time for restart to eating significantly in comparison with the non-stress group [F (4, 30) = 172.81, $P \leq 0.001$]. Lidocaine administration in to the right and left sides of the CeA augmented the stress effect and resulted in increased delay time in this groups significantly compared with the sham group [$P \leq 0.001$]. But interestingly bilateral inactivation of this nucleus leaded to reversing this effect and significant reduction in delay time for restart eating in proportion to sham group [$P \leq 0.001$].

Effects of acute stress on plasma glucose concentration in central amygdala inactivated rats

Our experiments revealed that acute stress induced elevation of the glucose concentration in female rats significantly compared with non-stressed group [F (4, 25) =23.92, $P \leq 0.001$]. According to Fig. 5, transient inactivation of the left [$P \leq 0.05$] and both sides [$P \leq 0.01$] of the central amygdala by lidocaine

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caused to significant increase in glucose concentration compared with the sham group. (Fig. 4C).

Discussion

The purpose of this study was to investigate the role of the central amygdala inactivation during acute stress on the hormonal and metabolic responses in this regard in female rats. The key findings were the increase of the plasma corticosterone, glucose and progesterone levels and elevation of the plasma estradiol after inhibition of the CeA. Furthermore, the glucose increment is exaggerated by transient inactivation of the left and bilateral sides of the CeA. Also, in the metabolic view of this study it is indicated that food intake and delay to eating time increments after stress induction. Transient inactivation of the right and/or left side(s) of the CeA caused to significant decrease in the water intake and increase in the delay time to onset of eating. In addition, bilateral inactivation of the CeA that induced significant reduction in delay time to onset of eating, also in among other important finding of this study.

Some researches in rodents revealed that the CeA is involved in adrenocortical stress activation and indicated its important role in the increase of the adrenocorticotropin gene and protein expression and corticosterone release during stress (Roozendaal et al., 1991; Van de Kar et al., 1991). In addition, it is revealed that there are the glucocorticoid receptors on the CeA neurons in which binding of glucocorticoids to these receptors may play a role in the anxiety and pain related behavior (Cahill, 2003; Myers and Greenwood-Van Meerveld, 2007). These findings revealed that CeA is involved directly in stress responses modulations (van der Kooy et al., 1984; Gray et al., 1989). Tract tracing studies have suggested that CRF neurons in the CeA send their axons to the bed nucleus of the stria terminalis, lateral hypothalamus, midbrain central gray, raphe nuclei, parabrachial region and also directly to the dopaminergic, noradrenergic and serotonergic neurons. Indeed, that CRF neurons of mentioned areas project back their axons to the CRF neurons in the central amygdala (Gray, 1993). It is revealed that amygdala responsiveness dependent on the type, period and duration of the stress (Wilson et al., 2015). According to previous studies, acute stress causes a rapid, significant and short-lasting increase in CeA glutamate in rats (Reagan et al., 2012), whereas repeated and chronic stress leads to decrease of extracellular CeA glutamate (Grillo et al., 2015). So, it can be concluded that different fluctuations in CeA glutamate output is the reason of modulatory effect of this nucleus on responses to stress. In the present study, however, acute stress may lead to increment in glutamate output from CeA; we didn't observe the expected effect of this nucleus on the increase of corticosterone and progesterone in response to stress.

Previous researches have revealed that acute physical or psychological stress leads to increase in plasma noradrenaline, adrenaline, corticosterone and glucose levels in the male rats (Van de Kar and Blair, 1999; Zardooz et al., 2010). Consistent with these studies in male rats, our results indicated that also this is true for female rats as well and our results indicated that our equipment for stress induction (i.e. communication box) is suitable for stress induction in female rats which induced elevation of the plasma corticosterone and glucose after acute stress. The mechanism of the elevation of the plasma glucose after stress can be explained by the effect of acute stress on the plasma catecholamine elevation as a result of activation of the sympathoadrenal system beside corticosterone and then the catecholamine stimulation of the glycogenolysis and so increase of the basal metabolic rates and the productions of glucose (Teague et al., 2007).

Results of the present experiment indicated that acute stress caused to significant elevation in the progesterone concentration. Our result is in agreement with the previous study which is revealed that adrenal progesterone secretion was increased along with the corticosterone in response to acute stress to modulate certain stress response (Kalil et al., 2013). In present study we test the transient inactivation of the left, right and bilateral sides of the CeA separately for evaluation of the lateralization of this nucleus in response to acute stress. Results have shown that the left side of the nucleus has more prominent effect on the elevation of the plasma glucose concentration than right side. This observation maybe indicate modulatory role of the left side CeA in the regulation of the glucose in female rats during acute stress and also indicate dominant role of the left side with respect to the right side in

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control of this factor. Our results are in the agreement with those of previous studies that support functional lateralization in the CeA. For example, Baas et al., in 2004 through neuroimaging studies has indicated that the activation of the left side of the amygdala is involved predominantly in emotional processing, whereas other fMRI studies have revealed that the right amygdala can overtop in emotional memory formation. Furthermore, other researches have shown the lateralization of the CeA in relation to pain processing (Carrasquillo and Gereau, 2008; Ji and Neugebauer, 2009). Is this finding are among evidences for the hypothesis that the CRF neurons of the left side may send their axons to the other regions that are involved in the reducing the effects of the stress on the HPA axis? or not? It seems that further investigations in this regard are needed. One possible mechanism may be involved in the observed effects of the CeA inactivation, is that the activation of the GABAergic inhibitory system in the cells of the CeA may reduce the side effects of the stress (Nose et al., 1991; Sun and Cassell, 1993). The presence of the local GABAergic connections within the central amygdala have verified by the morphological (Sun and Cassell, 1993) and electrophysiological (Nose et al., 1991) studies. Also, it is well proven through CeA whole-cell recordings that CRF and norepinephrine caused to enhancement of the $GABA_A$ receptor inhibitory postsynaptic potentials (Nie et al., 2004; Kash and Winder, 2006). Therefore, activation of the GABAergic system within the CeA may leads to increased inhibition of the downstream regions that are involved to the certain response to acute stress (Koob, 2009). In the other hand, this is demonstrated that the CeA mainly innervates the caudal, lateral and medial parvocellular paraventricular nucleus (PVN) of the hypothalamus. These pathways could form the anatomical substrates for amygdaloid-CeA-modulation of neuroendocrine responses to stressors (Gray et al., 1989).

According to results, stress didn't induce any significant effect on the plasma estradiol concentration in female rats. Considering this point that estradiol level reaching peak levels during proestrus phase, so the absence of the changes in this hormone in present study wasn't surprising (Marcondes et al., 2002). Indeed, according to result, inhibition of bilateral sides of the CeA leaded to significant elevation in the plasma estradiol level. Previous studies confirm the presence of the estradiol receptors in the amygdala (Jacobs et al., 2015). In the other hand, it is revealed that CRF production in response to stress caused to reduction in the gonadal function (Iwasaki-Sekino et al., 2009). Considering that CeA is the one of the prominent area for CRF production in response to stress (Iwasaki-Sekino et al., 2009). So, it may be suggested that this nucleus by increasing the CRF production in response to acute stress caused to suppress of ovarian function and reduced estradiol production.

In other part of the study, experiments revealed that acute stress caused to increased food consumption in female rats, this finding is in opposite to the stress effect on the food intake in male rats which was observed in the other studies (Gluck, 2006; Hooshmandi et al., 2011; Nasihatkon et al., 2014). The discrepancy may be due to the CRH level differences in male and female rats in the paraventricular nucleus of hypothalamus. Previous experiments revealed that CRF concentration is higher in the female versus male rats (Handa et al., 1994; Young, 1995). Indeed, our data showed that inhibition of the right, left and both sides of the central amygdala didn't cause to any more effect than stress effect on the food intake. However, in the other study it is revealed that lesion induction to the amygdala and exposure to swimming as physical and immobilization as a psychological types of chronic stress resulted in a significant decrease in the food intake (Ganaraja and Jeganathan, 2003). This observation may be explained by the difference between CeA role in the chronic stress in comparison with the acute stress (Watts, 2005; Russell et al., 2010). Also, between-sex differences in amygdala size and its receptor distribution may be explained this discrepancy (Hines et al., 1992; Ziabreva et al., 2003). The next part of this experiment is about the CeA involvement in the water intake. Stress alone has no significant effect on the amount of the water consumption. Whereas, the absence of the right and left side of CeA separately, caused to reduced water intake in comparison with non-stress and also sham groups significantly. In agreement with our results, in previous studies it is shown that unilateral lesions to CeA decreased the water intake (Ganaraja and Jeganathan, 2003). In previous researches it is well demonstrated that the CeA mainly innervates the parvocellular paraventricular nucleus of the

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hypothalamus (Gray et al., 1989). It is revealed that parvocellular neurons in the PVN provide vasopressin to the pituitary portal circulation which is responsible for the HPA axis regulation (Aguilera, 2011). It seems that, CeA especially in left side by using this pathway and then induction of the vasopressin release cause to thirst sensation and then increase the water intake in response to the stressors. Whereas, bilateral inactivation of this nucleus resulted in reversing this effect and significant difference with sham group that was seen in unilateral inhibition is abolished in the bilateral inactivation. It may be concluded that however inhibition of the each side separately leaded to significant increase in the water intake but the interaction between two sides of this nucleus have shown no significant difference with sham group in water intake. So, the presence of both sides of the CeA results in less increase in the water intake during stress relative to the each side separately. Our results indicated that acute stress resulted in increased delay times to start eating (anorexia). This observation is consistent with the previous studies which have shown that the anorexia is the common response to the acute stress (Ciccocioppo et al., 2003; Roman et al., 2012). CRF family of peptides which have known as a regulator of the stress-response, generally indicate inhibitory effects on the appetite and digestion in order to transport energy from parasympathetic need to arousal and sympathetic requirements in the stress conditions. Because from an evolutionary point energetic cost for prolonged anorexia is considered (Carr and Lovejoy, 2015). It seems that in the present study inhibition of the right and left sides of the CeA unilaterally leads to augmented CRF release from the other side of this nucleus and so exacerbated the anorexia effect of stress that was seen in this experiment and delay time for restart the eating increased significantly compared with sham group. This increase in delay time may indicate the inhibitory effects of CRF on appetite. Interestingly when bilateral sides of the CeA were inactivated, delay time to restart eating has decreased even less than sham group. So it may be suggested that inhibition of two sides of CeA in the same time has important role in reduction of CRF release from these nuclei and then caused to the reduction in delay time to restart eating during acute stress in female rats.

Conclusion

Results of the present study suggest that the central nucleus of the amygdala has a prominent role in regulation of the hormonal and metabolic responses to the acute stress in female rats. In conclusion, these findings are good evidence that left side of central amygdala can minimize the stress induced changes in hormonal and metabolic functions during an exposure to acute stress. Therefore, in the future studies more evaluation of the left CeA for the reduction of the side effects of stress is suggested. However, further experiments are needed to clarify the exact nature of this modulation and determine precise role of this nucleus and its interaction among other nuclei of amygdala and other brain areas in the regulation of homeostasis.

Acknowledgments

This work was supported by a grant from Neuroscience Research Center, Baqyiatallah (a.s.) University of Medical Sciences.

Conflict of interest

There is no conflict of interest in this article.

References

Aguilera G. Hpa axis responsiveness to stress: Implications for healthy aging. Exp Gerontol 2011; 46: 90-5.

Baas D, Aleman A, Kahn RS. Lateralization of amygdala activation: A systematic review of functional neuroimaging studies. Brain Res Brain Res Rev 2004; 45: 96-103.

Burchfield SR. The stress response: A new perspective. Psychosom Med 1979; 41: 661-72.

Cahill L. Sex- and hemisphere-related influences on the neurobiology of emotionally influenced memory. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27: 1235-41.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 2002; 26: 321-52.

Carr JA, Lovejoy DA. Energy metabolism and behavior in the corticotropin-releasing factor family of peptides. Front Neurosci 2015; 9: 122.

Carrasquillo Y, Gereau RW 4th. Hemispheric lateralization of a molecular signal for pain modulation in the amygdala. Mol Pain 2008; 4: 24.

Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. Annu Rev Physiol 2005; 67: 259-

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84.

Ciccocioppo R, Fedeli A, Economidou D, Policani F, Weiss F, Massi M. The bed nucleus is a neuroanatomical substrate for the anorectic effect of corticotropin-releasing factor and for its reversal by nociceptin/orphanin fq. J Neurosci 2003; 23: 9445-51.

Esmaeili MH, Sahraei H, Ali-Beig H, Ardehari-Ghaleh M, Mohammadian Z, Zardooz H, et al. Transient inactivation of the nucleus accumbens reduces both the expression and acquisition of morphine-induced conditioned place preference in rats. Pharmacol Biochem Behav 2012; 102: 249-56.

Ganaraj B, Jeganathan PS. Involvement of basolateral nucleus & central nucleus of amygdala in the regulation of ingestive behaviour in rat. Indian J Med Res 1998; 108: 98-103.

Ganaraja B, Jeganathan PS. Alterations in the ingestive behaviour of rats following unilateral lesions in the cea & bla. Indian J Med Sci 2003; 57: 383-6.

Gluck ME. Stress response and binge eating disorder. Appetite 2006; 46: 26-30.

Goldstein LE, Rasmusson AM, Bunney BS, Roth RH. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. J Neurosci 1996; 16: 4787-98.

Gray TS. Amygdaloid crf pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Ann N Y Acad Sci 1993; 697: 53-60.

Gray TS, Carney ME, Magnuson DJ. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible role in stress-induced adrenocorticotropin release. Neuroendocrinology 1989; 50: 433-46.

Grillo CA, Risher M, Macht VA, Bumgardner AL, Hang A, Gabriel C, et al. Repeated restraint stress-induced atrophy of glutamatergic pyramidal neurons and decreases in glutamatergic efflux in the rat amygdala are prevented by the antidepressant agomelatine. Neuroscience 2015; 284: 430-43.

Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. Horm Behav 1994; 28: 464-76.

Hines M, Allen LS, Gorski RA. Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. Brain Res 1992; 579: 321-6.

Hooshmandi Z, Rohani AH, Eidi A, Fatahi Z, Golmanesh L, Sahraei H. Reduction of metabolic and behavioral signs of acute stress in male wistar rats by saffron water extract and its constituent safranal. Pharm Biol 2011; 49: 947-54.

Iwasaki-Sekino A, Mano-Otagiri A, Ohata H, Yamauchi N, Shibasaki T. Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mrna expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. Psychoneuroendocrinology 2009; 34: 226-37.

Jacobs EG, Holsen LM, Lancaster K, Makris N, Whitfield-Gabrieli S, Remington A, et al. 17beta-estradiol differentially regulates stress circuitry activity in healthy and depressed women. Neuropsychopharmacology 2015; 40: 566-76.

Ji G, Neugebauer V. Hemispheric lateralization of pain processing by amygdala neurons. J Neurophysiol 2009; 102: 2253-64.

Kalil B, Leite CM, Carvalho-Lima M, Anselmo-Franci JA. Role of sex steroids in progesterone and corticosterone response to acute restraint stress in rats: Sex differences. Stress 2013; 16: 452-60.

Kash TL, Winder DG. Neuropeptide y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. Neuropharmacology 2006; 51: 1013-22.

Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, et al. Stressful life events, genetic liability, and onset of an episode of major depression in women. Am J Psychiatry 1995; 152: 833-42.

Kitay JI. Sex differences in adrenal cortical secretion in the rat. Endocrinology 1961; 68: 818-24.

Koob GF. Brain stress systems in the amygdala and addiction. Brain Res 2009; 1293: 61-75.

Koolhaas JM, Meerlo P, De Boer SF, Strubbe JH, Bohus B. The temporal dynamics of the stress response. Neurosci Biobehav Rev 1997; 21: 775-82.

Krizanova O, Babula P, Pacak K. Stress, catecholaminergic system and cancer. Stress 2016; 19: 419-28.

Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: Some helpful considerations. Braz J Biol 2002; 62: 609-14.

Merlo Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, et al. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci 1995; 15: 5439-47.

Moaddab M, Haghparast A, Hassanpour-Ezatti M. Effects of reversible inactivation of the ventral tegmental area on the acquisition and expression of morphine-induced conditioned place preference in the rat. Behav Brain Res 2009; 198: 466-71.

Myers B, Greenwood-Van Meerveld B. Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. Am J Physiol Gastrointest Liver Physiol 2007; 292: G1622-9.

Nasihatkon ZS, Khosravi M, Bourbour Z, Sahraei H, Ranjbaran M, Hassantash SM, et al. Inhibitory effect of nmda receptors in the ventral tegmental area on hormonal and eating behavior responses to stress in rats. Behav Neurol 2014; 2014: 294149.

Nie Z, Schweitzer P, Roberts AJ, Madamba SG, Moore SD, Siggins GR. Ethanol augments gabaergic transmission in the central amygdala via crf1 receptors. Science 2004; 303: 1512-4.

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Nose I, Higashi H, Inokuchi H, Nishi S. Synaptic responses of guinea pig and rat central amygdala neurons in vitro. J Neurophysiol 1991; 65: 1227-41.

Pacak K, Palkovits M. Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. Endocr Rev 2001; 22: 502-48.

Reagan LP, Reznikov LR, Evans AN, Gabriel C, Mocaer E, Fadel JR. The antidepressant agomelatine inhibits stress-mediated changes in amino acid efflux in the rat hippocampus and amygdala. Brain Res 2012; 1466: 91-8.

Roman CW, Lezak KR, Kocho-Schellenberg M, Garret MA, Braas K, May V, et al. Excitotoxic lesions of the bed nucleus of the stria terminalis (bnst) attenuate the effects of repeated stress on weight gain: Evidence for the recruitment of bnst activity by repeated, but not acute, stress. Behav Brain Res 2012; 227: 300-4.

Roozendaal B, Koolhaas JM, Bohus B. Attenuated cardiovascular, neuroendocrine, and behavioral responses after a single footshock in central amygdaloid lesioned male rats. Physiol Behav 1991; 50: 771-5.

Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. J Neurosci 2010; 30: 6106-15.

Sun N, Cassell MD. Intrinsic gabaergic neurons in the rat central extended amygdala. J Comp Neurol 1993; 330: 381-404.

Teague CR, Dhabhar FS, Barton RH, Beckwith-Hall B, Powell J, Cobain M, et al. Metabonomic studies on the physiological effects of acute and chronic psychological stress in sprague-dawley rats. J Proteome Res 2007; 6: 2080-93.

Toleikis PM, Godin DV. Alteration of antioxidant status in diabetic rats by chronic exposure to psychological stressors. Pharmacol Biochem Behav 1995; 52: 355-66.

Valentino RJ, Bangasser D, Van Bockstaele EJ. Sex-biased stress signaling: The corticotropin-releasing factor receptor as a model. Mol Pharmacol 2013; 83: 737-45.

Valles A, Marti O, Garcia A, Armario A. Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. Am J Physiol Regul Integr Comp Physiol 2000; 279: R1138-44.

Van de Kar LD, Blair ML. Forebrain pathways mediating stress-induced hormone secretion. Front Neuroendocrinol 1999; 20: 1-48.

Van de Kar LD, Piechowski RA, Rittenhouse PA, Gray TS. Amygdaloid lesions: Differential effect on conditioned stress and immobilization-induced increases in corticosterone and renin secretion. Neuroendocrinology 1991; 54: 89-95.

van der Kooy D, Koda LY, McGinty JF, Gerfen CR, Bloom FE. The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. J Comp Neurol 1984; 224: 1-24.

Viau V, Bingham B, Davis J, Lee P, Wong M. Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. Endocrinology 2005; 146: 137-46.

Watts AG. Glucocorticoid regulation of peptide genes in neuroendocrine crh neurons: A complexity beyond negative feedback. Front Neuroendocrinol 2005; 26: 109-30.

Williams LM, Das P, Liddell BJ, Olivieri G, Peduto AS, David AS, et al. Fronto-limbic and autonomic disjunctions to negative emotion distinguish schizophrenia subtypes. Psychiatry Res 2007; 155: 29-44.

Wilson MA, Grillo CA, Fadel JR, Reagan LP. Stress as a one-armed bandit: Differential effects of stress paradigms on the morphology, neurochemistry and behavior in the rodent amygdala. Neurobiol Stress 2015; 1: 195-208.

Young EA. The role of gonadal steroids in hypothalamic-pituitary-adrenal axis regulation. Crit Rev Neurobiol 1995; 9: 371-81.

Zardooz H, Rostamkhani F, Zaringhalam J, Faraji Shahrivar F. Plasma corticosterone, insulin and glucose changes induced by brief exposure to isoflurane, diethyl ether and co2 in male rats. Physiol Res 2010; 59: 973-8.

Ziabreva I, Poeggel G, Schnabel R, Braun K. Separation-induced receptor changes in the hippocampus and amygdala of octodon degus: Influence of maternal vocalizations. J Neurosci 2003; 23: 5329-36.