



Original Article

Acute physical and psychological stress effects on visceral hypersensitivity in male rat: role of central nucleus of the amygdala



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ARTICLE INFO

Article history:

Received 19 November 2016

Accepted 21 December 2016

Available online 27 February 2017

Keywords:

Abdominal withdrawal reflex

Amygdala

Physical stress

Psychological stress

Visceral hypersensitivity

ABSTRACT

Objective: The aim of this study was to investigate the effects of acute physical and psychological stress and temporary central nucleus of the amygdala (CeA) block on stress-induced visceral hypersensitivity.

Methods: Forty two male Wistar rats were used in this study. Animals were divided into 7 groups ($n = 6$); 1 – Control, 2 – physical stress, 3 – psychological stress, 4 – sham, 5 – lidocaine, 6 – lidocaine + physical stress and 7 – lidocaine + psychological stress. Stress induction was done using a communication box.

Results: Abdominal withdrawal reflex (AWR) score was monitored one hour after stress exposure. AWR score significantly heightened at 20, 40 and 60 mmHg in the psychological stress group compared with control ($p < 0.05$), while, it was almost unchanged in other groups. This score was strikingly decreased at 20, 40 and 60 mmHg in lidocaine + psychological stress group compared with psychological stress with no tangible response on physical stress. Total stool weight was significantly increased in psychological stress group compared with control (0.72 ± 0.15 , 0.1 ± 0.06 g) ($p < 0.05$), but it did not change in physical stress compared to control group (0.16 ± 0.12 , 0.1 ± 0.06 g) ($p < 0.05$). Concomitant use of lidocaine with stress followed the same results in psychological groups (0.18 ± 0.2 , 0.72 ± 0.15 g) ($p < 0.05$), while it did not have any effect on physical stress group (0.25 ± 0.1 , 0.16 ± 0.12 g) ($p < 0.05$).

Conclusions: Psychological stress could strongly affect visceral hypersensitivity. This effect is statistically comparable with physical stress. Temporary CeA block could also reduce visceral hypersensitivity post-acute psychological stress.

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<http://dx.doi.org/10.1016/j.jcol.2016.12.006>

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Efeitos agudos do estresse físico e psicológico na hipersensibilidade visceral em rato macho: papel do núcleo central da amígdala

R E S U M E N

Palavras-chave:

Reflexo de retirada abdominal
Amígdala
Estresse físico
Estresse psicológico
Hipersensibilidade visceral

Objetivo: O objetivo desse estudo foi investigar os efeitos do estresse físico e psicológico agudo e bloqueio temporário do núcleo central da amígdala (CeA) na hipersensibilidade visceral induzida por estresse.

Métodos: Quarenta e dois ratos Wistar machos foram empregados nesse estudo. Os animais foram divididos em 7 grupos (n = 6): 1 – Controle, 2 – estresse físico, 3 – estresse psicológico, 4 – simulacro, 5 – lidocaína, 6 – lidocaína + estresse físico e 7 – lidocaína + estresse psicológico. A indução do estresse foi feita com o uso de uma caixa de comunicação.

Resultados: O escore do reflexo de retirada abdominal (RRA) foi monitorado uma hora depois da exposição ao estresse. O escore RRA aumentou significativamente a 20, 40 e 60 mmHg no grupo de estresse psicológico versus controle (p < 0,05), enquanto que praticamente permaneceu inalterado nos demais grupos. Esse escore diminuiu drasticamente a 20, 40 e 60 mmHg no grupo de lidocaína + estresse psicológico versus estresse psicológico, sem resposta tangível no estresse físico. O peso total das fezes aumentou significativamente no grupo de estresse psicológico versus controle (0,72 ± 0,15, 0,1 ± 0,06 g) (p < 0,05), mas não houve mudança no grupo de estresse físico versus controle (0,16 ± 0,12, 0,1 ± 0,06 g) (p < 0,05). O uso simultâneo da lidocaína com o estresse acompanhou os mesmos resultados nos grupos psicológicos (0,18 ± 0,2, 0,72 ± 0,15 g) (p < 0,05), enquanto que não foi observado qualquer efeito no grupo de estresse físico (0,25 ± 0,1, 0,16 ± 0,12 g) (p < 0,05).

Conclusões: O estresse psicológico pode afetar fortemente a hipersensibilidade visceral. Esse efeito é estatisticamente comparável com o estresse físico. Um bloqueio temporário do CeA também pode reduzir a hipersensibilidade visceral pós-estresse psicológico agudo.

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Introduction

Visceral hypersensitivity is now a common hypothesis used to explain the painful symptoms in patients suffering from irritable bowel syndrome (IBS). IBS is a prevalent gastrointestinal disorder characterized by abdominal pain and bowel habit changes with unexplored etiology.¹ Almost 22% of the population experiences IBS symptoms.² Difficulty in diagnosis and inadequate good treatment together with high social and medical costs changed IBS into a social concern.^{3,4}

Psychological and physical stressors have been identified as important factors in IBS pathophysiology.^{5,6} Researchers have found a relationship between central pathways of stress and anxiety and peripheral mechanisms modulating gastrointestinal sensitivity. Amygdala, especially the central nucleus of amygdala (CeA), which has a pivotal role on this relationship; mainly affects endocrine and autonomic reply to stress and causes visceral hypersensitivity.⁷ Visceral hypersensitivity is commonly examined via different forms among which communication box seems to be an agreeable tool for concomitant study of physiological and behavioral changes under psychological and physical stresses.⁸

However, to the best of our knowledge, this is the first study which focuses on rat visceral hypersensitivity post induction in the communication box environment. Therefore, the aim of the current study was to investigate the role of CeA block in visceral hypersensitivity response post communication box induced physical and psychological stress model in male rats.

Materials and methods

Animals

Forty two male Wistar rats (250–325 g) purchased from physiology department of Tehran University of Medical Sciences (TUMS). Animals were kept under standard conditions of 12-h:12-h light/dark cycle with unlimited access to the standard rat chow and water. All experiments were performed upon TUMS ethical committee approval between 8 am up to 3 pm every day.

Experimental protocol

Experiments were performed on seven equally assigned animal groups (n = 6) as per the following classification:

1. Control; animals placed in stress box 15 min without receiving electric foot shock.
2. Physical stress; animals placed in stress box and received 5 s electrical shock (2 mA), then 55 s rest for 15 min.
3. Psychological stress; animals placed in the safe compartments for 15 min without receiving foot shock and then received visual, auditory and olfactory sensation cues from the neighboring animal,
4. Sham; animals received bilateral microinjections of normal saline (0.5–1 µl) into central nucleus of the amygdala

(CeA) 5 min before placing into the stress box followed by the same procedure as group 1.

5. Lidocaine; animals received bilateral microinjections of lidocaine (0.5–1 μ l, 2%) into CeA
6. Physical stress + lidocaine; animals received bilateral microinjections of lidocaine into CeA 5 min before placing into the stress box followed by the same procedure as group 2.
7. Psychological stress + lidocaine; animals received bilateral microinjections of lidocaine into CeA 5 min before placing into the stress box followed by the same procedure as group 3.

Stereotaxic surgery

Animals anesthetized first with a mixture of ketamine (50 mg/kg, ip) and xylazine (5 mg/kg, ip) and placed in a stereotaxic apparatus. The skull was clean shaved and a midline incision made. Connective tissues were then removed to have the best access to lambda bregma landmarks. Two holes were made with a micro drill over the skull according to rat brain anatomical atlas (paxinos) coordinates (2.5 mm posterior to the bregma, 4.4 mm bilateral to midline and 8 mm from the top). Two 23-gauge stainless steel guide cannulas were bilaterally lowered until their tips stood 2 mm above CeA and fixed to the skull surface with dental cement. Cannula sites were confirmed via methylene blue color injection and tissue section studies. Upon completion, animals backed to their cages for a week recovery before handling stress accommodation.⁹ Normal saline (0.5–1 μ l) and lidocaine (0.5–1 μ l, 2%)^{10,11} delicately injected with a Hamilton syringe connected to a 30-gauge cannula extended 2 mm below the guide cannula tip. Lidocaine acted as temporary blocker of both neurons and tracts in the amygdala region.¹¹ Injections were made over 1 min to allow maximal diffusion. The cannula was also slowly withdrawn during the same time period.⁹

Body temperature kept at 37°C with a homoeothermic heating pad.

Stress box and electrical shock device

Communication box apparatus was designed for stress stimulus application. The apparatus consists of nine compartments of 50 cm \times 16 cm \times 16 cm, separated by transparent plastic walls. To apply psychological stress, each rat is individually placed into one compartment and isolated from others by transparent walls with no physical contact, but free to receive visual, auditory and olfactory sensation cues from the neighboring rat. The floor of each compartment was covered with metal grids made up of 5 mm diameter stainless steel rods placed 1.3 cm apart connected to an electric shock generator (current of 2 mA) (Borj Sanat Co., Iran, sensitivity 0.1–10 mA) to apply foot shock with 5 s duration and 55 s interval.¹² Four compartments grids were insulated with plastic rolls to serve as shock-free compartments for psychological stress group.¹³

Total stool weight measurement

Each rat backed to an individual cage immediately after stress exposure for 30 min with free access to standard chow and

water. Feces were collected, weighed and reported as total stool weight.¹²

Behavioral tests

Visceral hypersensitivity was monitored 1 hour after stress exposure using visual observation of animal response to graded colorectal distention (CRD), as previously described.^{14,15} Animals inhaled diethyl ether inhalation under a glass bell for a light anesthesia. A 5 cm balloon made up of surgical glove finger attached to a tygon tube, inserted into the anal canal and advanced 8 cm into the descending colon and fixed to the animal tail with an adhesive tape. The tube end attached to a sphygmomanometer via a Y-connector. Rats were then recovered from anesthesia and allowed to adapt for about 30 min. CRD was carried out through rapidly inflating the balloon to different pressure amount including 20, 40, 60 and 80 mmHg for a 20 s followed by 2 min rest. Behavioral responses to CRD were monitored by an observer blind to the experiment and recorded as abdominal withdrawal reflex (AWR) score as follows: 0, no behavioral response; 1, brief head movement followed by immobile status; 2, abdominal muscle contraction; 3, abdominal wall lifting; 4, body arching and pelvic structures lifting.

Determination of plasma corticosterone level

At the end of the experiments, animals were anesthetized with diethyl ether. Blood samples were collected by cardiac puncture and kept in EDTA coated tubes for further plasma separation. Samples stored at –70°C till morning assay. Corticosterone ELISA kit (DRG, Germany) was used to determine corticosterone level.

Histology

At the end of the experiment, injection sites were evaluated for accuracy with methylene blue microinjections (0.25 μ L/each side). Animals were then decapitated under diethyl ether inhalation. Brains were removed and fixed in formalin (10%) solution. Coronal sections (50 μ m) were also prepared and examined for CeA injection site.¹⁶

Statistical analysis

Data represented as mean \pm SEM. Statistical analysis was performed by SPSS software, version 19.0. Nonparametric Friedman test used to assess AWR scores at respective pressures within each group. Averaged AWR score at each CRD pressure were compared among groups using Kruskal–Wallis one-way analysis of variance. Hormone levels and total stools weight were compared with analysis of variance (ANOVA) followed by Tukey post hoc test. $p < 0.05$ was considered as significant statistical level.

Results

Acute physical and psychological stresses and temporary CeA block effects on AWR scores were shown in (Fig. 1).

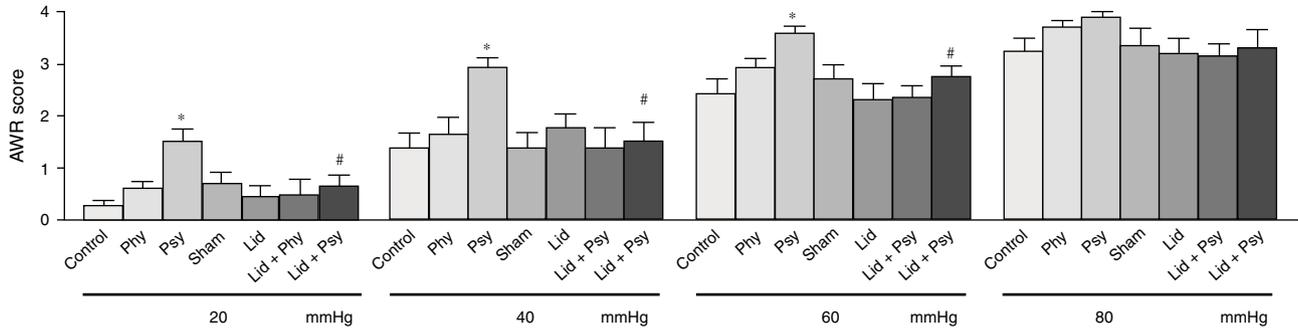


Fig. 1 – Effect of acute physical and psychological stresses and temporary central nucleus of amygdala (CeA) block on withdrawal reflex (AWR) scores. Control, physical (phy), psychological (psy), sham, lidocain (lid), lidocain + physical (lid + phy), lidocain + psychological (lid + psy) stresses. * $p < 0.05$ comparison of psy stress group with control and # $p < 0.05$ lid + psy with psy.

AWR scores significantly increased at 20, 40 and 60 mmHg in psychological stress group compared with control ($p < 0.05$). AWR scores in other groups were slightly increased at 20, 40, 60 and 80 mmHg compared with control ($p > 0.05$). These scores were significantly decreased at 20, 40 and 60 mmHg in lidocaine + psychological stress group compared with the psychological stress group. No significant differences were observed between AWR scores of lidocaine + physical stress compared with physical stress group.

Total stool weight post stress exposure in psychological stress group was significantly higher than control group (0.72 ± 0.15 , 0.1 ± 0.06 g), but it did not statistically change in physical stress compared to control group (0.16 ± 0.12 , 0.1 ± 0.06 g) (Fig. 2). Rats in psychological + lidocaine stress

group showed a significant decrease in total stool weight compared with psychological stress group (0.18 ± 0.2 , 0.72 ± 0.15 g). No significant difference was observed between total stool weights in lidocaine + physical group compared with physical one (0.25 ± 0.1 , 0.16 ± 0.12 g) (Fig. 2).

The effect of acute physical and psychological stresses and temporary CeA block on plasma corticosterone level were shown in (Fig. 3). Significant differences were seen in plasma corticosterone levels of control, physical and psychological stress groups (1258.17 ± 11.9 , 1723.50 ± 5.7 , 1700.50 ± 5.5 ng/ml) ($p < 0.05$). Plasma corticosterone level significantly decreased in lidocaine group compared with the sham animals (1235.50 ± 8.6 , 1622 ± 1.6 ng/ml) ($p < 0.05$).

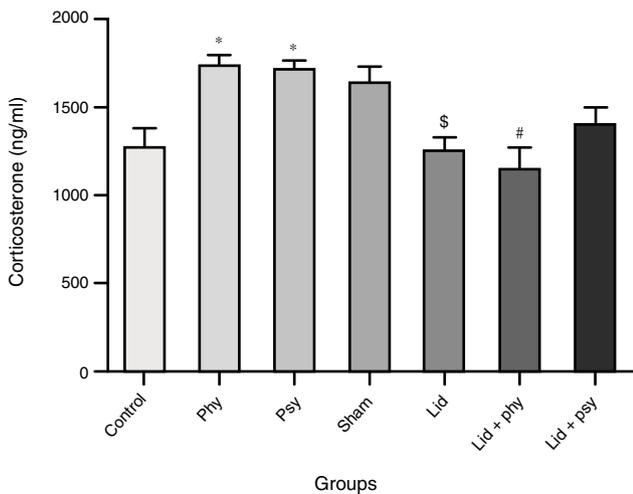


Fig. 2 – Effect of acute physical and psychological stresses and temporary central nucleus of amygdala (CeA) block on total stool weight post stress exposure during 30 min. Control, physical (phy), psychological (psy), sham, lidocain (lid), lidocain + physical (lid + phy), lidocain + psychological (lid + psy) stresses (Mean \pm SE, $n = 6$). * $p < 0.05$ comparison of psy stress groups with control and # $p < 0.05$ comparison of psy + lid stress with psy stress group.

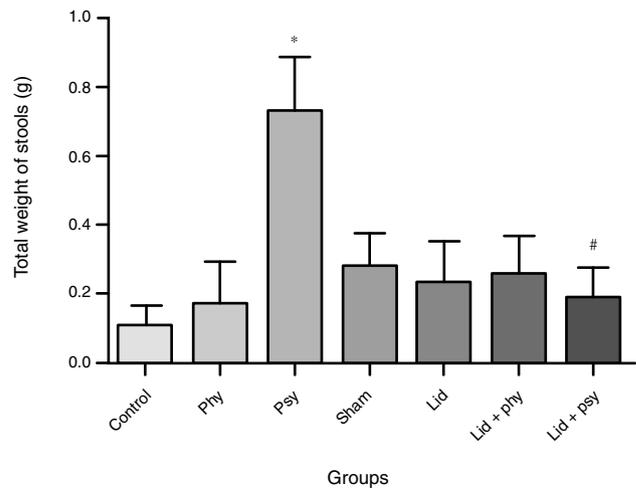


Fig. 3 – Effect of acute physical and psychological stresses and temporary central nucleus of amygdala (CeA) block on plasma corticosterone level. Control, physical (phy), psychological (psy), sham, lidocain (lid), lidocain + physical (lid + phy), lidocain + psychological (lid + psy) stresses (Mean \pm SE, $n = 6$). * $p < 0.05$ comparison of phy and psy stress groups with Control, \$lid with sham $p < 0.05$ and # $p < 0.05$ lid + phy, lid + psy with phy and psy.

Plasma corticosterone level was significantly decreased in lidocaine + physical group compared with physical group (1132.33 ± 13.6 , 1723.50 ± 5.7 ng/ml), whereas no significant changes were observed between corticosterone level of the lidocaine + psychological group compared with psychological group (1391.67 ± 9.9 , 1700.50 ± 5.5 ng/ml) (Fig. 3).

Discussion

In the present study, we investigated the acute physical and psychological stress effects on visceral hypersensitivity together with temporary CeA block response to stress-induced visceral hypersensitivity in male rat. One clinical study reported when IBS patient faced with acute stress, decreased significantly rectal threshold perception and pain. As a result of increased visceral sensitivity in these patients.¹⁷

Our results showed that acute psychological stress could significantly increase abdominal contraction responses to CRD especially at 20, 40, and 60 mmHg. This is in congruent with other preceding studies showing increased visceral sensitivity post-acute physical and psychological stresses.¹⁷⁻¹⁹

Some studies have shown limbic system involvement including amygdala in response to visceral stimulation,²⁰⁻²² also the techniques that examined brain activity have shown that in patients with IBS activity of limbic regions including the amygdala increased in response to rectosigmoid Stimulation.^{22,23}

Amygdala is a central integrator for emotion, nociception, emotional pain behavior, and emotional evaluation of sensory stimuli and is vital for fear and anxiety senses.^{20,24}

It consists of many nuclei and receives versatile information from different somatosensory, olfactory, auditory, visual, gustatory and visceral senses. The lateral nucleus (LA) is its main input of the amygdala while the central nucleus (CeA) acts as the output. CeA projects mainly to cortical, hypothalamic, and brain stem regions.²⁵ In this study we temporary blocked the output tracks and assessed stress-induced colonic hypersensitivity after temporary CeA block, especially at lower pressures. In addition, we noted that temporary CeA block could decrease visceral hypersensitivity clearly among animals exposed to psychological stress.

Sever stress is outcomes life car and predispose to diseases such as IBS. Stress first activates hypothalamic-pituitary-adrenal (HPA) axis. This causes hypothalamic corticotropin releasing hormone (CRH) secretion at the next step and finally pituitary adrenocorticotropin (ACTH) secretion and corticosterone release.²⁶

In this study, plasma corticosterone level was significantly increased in physical and psychological stress groups in comparison to control. These results are in congruent with previous studies indicating increased level of plasma corticosterone as the most important signs of physical & psychological stress.^{27,28}

Stimulation of CeA has been shown to ease the HPA axis activation,^{29,30} while its lesions could block the axis and plasma corticosterone secretion.³¹

In this study increased plasma corticosterone level post physical stress was blocked by bilateral CeA lidocaine microinjections, but it was unaffected post psychological stress. This

notable finding may raise the probability that an alternative modulatory pathway except that CeA is involved. Such a pathway may relate either to another central integration center or to shifted peripheral responses of visceral stimuli. Expression of different subtypes of CRH receptors with versatile regulatory response in gut functions may also explain diverse response post psychological stress application. Some studies have shown expression of two CRH receptor subtypes with quite opposite gut functions post maternal separation.³²

Studies have depicted that autonomic imbalance including low vagal tone and increased sympathetic activity may also change visceral sensitivity.³³ Our data showed that the autonomic system may be more contributed in visceral perception to luminal distension than the HPA axis since colonic hypersensitivity decreased after temporary CeA block, but increased plasma corticosterone level was unaffected.

In the present study, we also assessed the acute physical and psychological stress effects on total stool weight and found a significant increase post psychological stress application. This finding is in agreement with previous results showing that both stressors could enhance colonic motor activity and defecation.^{12,34} However, total stool weight was not significantly increased in animals exposed to physical stress.

Previous studies stated that major CeA output to solitary tract nucleus and neurons in dorsal motor nucleus of the vagus regulates colonic motility.^{35,36} We observed that temporary CeA block could significantly decrease total stool weight in rats exposed to psychological stress.

In conclusion, the present study showed the effects of acute physical and psychological stress together with temporary CeA block on visceral hypersensitivity, plasma corticosterone level and, total stool weight in studied rats. We also depicted that psychological stress is much more effective than physical stress on visceral hypersensitivity and total stool weight, whereas both stress types have similar effects on plasma corticosterone level.

Temporary CeA block could decrease plasma corticosterone level in acute physical stress, with no effect on plasma corticosterone level in acute psychological stress.

Visceral sensitivity and defecation clearly reduced after temporary CeA block in acute psychological stress especially at lower pressures but remained unchanged in acute physical stress.

Visceral hypersensitivity is common in IBS patients and stress is one of the factors reinforcing visceral sensitivity. In conclusion, visceral hypersensitivity is an interesting topic for researchers and a target for the discovery of a novel drug for the treatment of IBS.³⁷

Our findings highlighted the importance of psychological stress on visceral hypersensitivity and may help in evaluating the patients suffering from IBS under psychological stress or designing therapeutical modalities with more emphasis on the autonomic dysregulation.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

This research was supported by Tehran University of Medical Sciences.

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