Angiotensin II type 1 receptor blocker losartan attenuates locomotor, anxiety-like behavior, and passive avoidance learning deficits in a sub-chronic stress model

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Introduction

Stress is indicated as a risk factor for mental and psychological disorders, including depression and anxiety disorders (1, 2), which leads to disturbance of brain homeostasis (3). Stress is associated with defensive behaviors that are involved in emotional states of anxiety (4). Previous studies reported that stress has a considerable impact on impairing exploration and locomotor activities (5).

However, responding to stress depends on various factors including, ability to adapt to stress, which is related to sex, age, genetics, duration, and environmental influences (6). Therefore, it should be noted that depending on the type of stress different results are expected. Forced swim stress is one of the effective tests for the interpretation of anxiety and depression-like behavior. Hence, in the present work, it was used to cause psychological disorders (7).

In terms of detrimental effects of stress, anxious conditions have different complex effects (e.g. facilitating, impairing, and neutral) on memory (6). Although this demonstrates the multifaceted effects of stress on memory processing, numerous studies have emphasized the cognitive deficits and emotional processes related to memory caused by stress (8). Stress-induced memory deficits have raised many clinical efforts to develop medications, which appear to have some benefit on cognitive functions. Losartan is a widely used antihypertensive drug in patients, interestingly, recent evidence considered it as an effective drug in the treatment of memory defects (9). Thus, we decided to examine these claims by using passive avoidance test to evaluate memory processes and losartan impacts on it.

In the recent decades, scientists showed interest in understanding the interactions between stress and pain. These data suggest that the nature, duration, and intensity of the stressor are key factors of the effects of stress on pain (10, 11). Pain is a very subjective phenomenon, difficult to quantify but at the same time can be problematic if left untreated (12).

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It is important to note that the stimulatory effect of psychological stress induces physiological activity on pain sensitivity. Indeed, chronic exposure to physical or psychological stressors results in stress-induced hyperalgesia (SIH) (10).

The changes in pain sensitivity seem to be modulated by the types of stimuli used for inducing stress. Several theoretical frameworks have been proposed (13). In our study, we used forced swim stress (FSS). Hyperalgesia in rats exposed to repeated swim-stress has been demonstrated (14), which confirms FSS as a useful model for studying SIH. In addition, recent studies have shown that the administration of losartan plays a role in modulation of the pain system (15, 16). Thus, this study was undertaken to evaluate the effects of losartan on pain perception in rats after receiving FSS.

In general, the present study was designed to investigate the advantages and drawbacks of losartan administration on stress symptoms, particularly its effects on anxiety behavior; memory processes, and pain.

Materials and Methods

Experimental animals

The experiments were performed on 64 male Wistar rats (180–220 g), which were obtained from the laboratory animals of the Neuroscience Research Center of Kerman Medical University and the study protocol was approved by The Animal Ethics Committee of this institution [code: EC/KNRC/89-169]. Rats were housed under standard laboratory conditions with a 12 hr light/ dark cycle (lights on at 07:00, off at 19:00) at a constant temperature (22±2 °C). Food and water were available ad libitum

Hot plate

The animals in each group were placed one by one on the hot plate for evaluation of pain sensitivity (with 19 cm diameter and 30 cm height), which was surrounded by a transparent Plexiglas chamber with an open top, the reaction time was noted at 30, 60, and 90 min intervals. Reaction time is the time from the onset of radiant heat (adjusted to yield baseline latencies of 2–4 sec) was applied to tail at 5–8 cm from the tip. The cut-off point as tail response sufficient to interrupt the tissue damage was established at 10 sec. The animals showing baseline latency times of less than 2 or more than 4 sec were excluded from the study. The latency times were determined in 15 min intervals. Antinociception was quantified as tail flick latency time (25).

Passive avoidance test

A shuttle-box apparatus was used for passive
avoidance test. The apparatus consisted of one lighted chamber and one dark chamber with grid door. Electrical shocks were transferred by a separated stimulator to the grid floor of the shuttle box. This test was performed for each rat for two consecutive days. In the first day, each rat was put in the device to habituate. After habituation, an acquisition trial was performed in which animals were initially placed in the lighted compartment and the door between the two compartments was opened. The initial latency for a rat to enter the dark compartment was measured. After entering the dark compartment, the door was closed and an electric foot-shock (0.5 mA, 50 Hz, 2 sec once) was delivered through the stainless steel rods. All animals examined, entered the dark compartment within 60 sec as cutoff latency in the training session, and received a foot-shock. Step-through latency (STL) for animals was recorded on the second day using the same paradigm, but without foot-shock (20, 26).

Corticosterone level

One day after swimming sessions (8:00 am), animals were anesthetized using carbon dioxide (CO$_2$) and were killed by decapitation. Trunk blood was collected and centrifuged at 2600 rpm for 20 min. The serum was refrigerated at −80 °C until the day of analysis. Samples were then analyzed blindly by an ELISA kit used specifically for rats.

Drugs and treatments

Animals were randomly divided into 8 groups, each group receiving one of the following treatments: normal saline (0.5 cc) [FS (pain and PA tasks subgroups) and SS (pain and PA tasks subgroups)], losartan (Ang II receptor blockade, 10 mg/kg, IP) [FS (pain and PA tasks subgroups) and SS (pain and PA tasks subgroups)] (27).

Data analysis

All data are reported as the mean ± SEM. The data of various groups (between groups) were compared using two way ANOVA followed by Tukey’s test for multiple comparisons. Two-way ANOVA was performed to compare a significant interaction of stress (sham or stress condition) × treatment (losartan or saline) for locomotor activity in rotarod, pain threshold in tail flick and hot plate, and procedure of learning in passive avoidance. A $P$-value of less than 0.05 was considered statistically significant.

Results

Effect of forced swim stress and losartan on locomotor and anxiety-related behaviors

The open-field test was conducted to examine locomotor activity and anxiety-related behaviors. In this test, stress altered total locomotor activity (Figure 1). Data showed no significant difference in velocity ($F$ (3, 28) = 0.49, $P$ > 0.05; Figure 1a) and total distance moved ($F$ (3, 28) = 0.68, $P$ > 0.05; Figure 1b) in all groups compared to the control group. A two way ANOVA revealed that stress significantly altered time spent in the center ($F$ (3, 28) = 3.62, $P$ < 0.01; Figure 1c). Forced swim rats stayed less in the center in comparison to sham rats (Ns + Sham and Losartan + Sham groups). Losartan + stress group

![Figure 1](image-url). Effect of stress and losartan on A) velocity, B) total distance moved, C) time spent in the center, D) time spent in perimeter area, E) grooming, and F) mobility in open field test. Results are expressed as mean±SEM (ANOVA test, for comparisons between groups; *$P$<0.05, **$P$<0.01 when compared to Ns+Sham group; x$P$<0.05, xx$P$<0.01 when compared to Losartan+Sham group; ##$P$<0.01 when compared to Ns+Stress group)
rats stayed more in the center of the open field compared to forced swim rats, indicating losartan decreased anxiety and stress behavior by enhancement of tendency to spend more time in the center area. The time spent in perimeter area in Ns + Stress group, were significantly higher than those in the Ns + Sham and Losartan + Sham groups (F (3, 28) = 2.8, P < 0.05; Figure 1d).

Stress had significant alternation in grooming (F (3, 28) = 1.26, P < 0.05; Figure 1e), which increased aggressive grooming compared to the sham groups. In line with our findings, some authors proposed that grooming behavior is linked with an anxiolytic state in rodents and partially explains anxiety states.

The mobility of the Ns + Stress group was increased when compared to Ns + Sham and Losartan + Sham groups (P < 0.01 in both cases). No differences, however, were found in other groups (F (3, 28) = 3.38, P > 0.05; Figure 1f).

Effect of swim stress and pretreatment with losartan on passive avoidance learning

FSS and pretreatment with losartan did not significantly change the number of shocks compared to the sham swim group (F (3, 28) = 0.18, P > 0.05; Figure 2a). Step through latency was recorded for all groups. Interestingly, latency to enter the dark compartment was decreased in the Ns + stress group compared with both Ns + sham and Losartan + sham groups (F (3, 28) = 3.12, P < 0.05, P < 0.001, respectively; Figure 2b). Whereas, Losartan + stress rats significantly (P < 0.01) showed less tendency toward the dark chamber compared to the Ns + stress group. It may indicate the positive effects of losartan on lowering the harmful impact of stress on memory.

As expected the total dark component in the Ns + stress group increased compared with their own sham group (P < 0.05). Meanwhile, Losartan + stress group showed a reduction in the time spent in the dark side when compared to Ns + stress group (F (3, 28) = 6.2, P < 0.001; Figure 2c).

Effect of swim stress and pretreatment with losartan on thermal pain thresholds

Hot plate test was performed to evaluate the pain sensitivity before and after the induction of stress. However, no significant differences were detected in any experimental group (Figure 3a). Thermal pain threshold was significantly increased in forced swim groups in the tail flick test compared to sham groups (F (3, 28) = 8.9, P < 0.001; Figure 3b), while no differences between other groups were observed and losartan could not compensate this effect of swim stress.

The effect of stress and losartan on corticosterone level

Plasma corticosterone levels in none of the non-FSS groups showed any changes during the experimental period. Two-way ANOVA showed a significant higher plasma corticosterone level in the FSS treated groups (Table 1). However, there were no significant differences by losartan supplementation. Exposing to losartan did not show any significant changes in plasma corticosterone levels in the Ns + Sham and Losartan + Sham groups.

Table 1. Plasma corticosterone level

| Condition            | Plasma corticosterone level (pg/ml) |
|----------------------|-------------------------------------|
| Ns + Sham            | 89.56±6.09                          |
| Losartan + Sham      | 95.33±1.34                          |
| Ns + Stress          | 118.14±7.19*                        |
| Losartan + Stress    | 106.28±13.08                        |

Changes in plasma corticosterone levels after FSS and losartan administration. Data are presented as means±SEM. Statistical analysis was performed by two-way ANOVA. *P < 0.05, when compared to Ns + Sham group; xxP < 0.05, when compared to Losartan + Sham group.

Figure 2. Effect of stress and losartan in passive avoidance test. A) shock number, B) step through latency, C) total dark component. Results are expressed as means±SEM (ANOVA test, Tukey’s test for comparisons between groups; *P < 0.05, **P < 0.01 when compared to Ns+Sham group; xxP < 0.05, xxxP < 0.001 when compared to Losartan+Sham group)

Figure 3. Effect of stress and losartan on pain in A) hot plate and B) tail flick tests. Results are expressed as means±SEM (ANOVA test for comparisons between groups; ***P < 0.001 when compared to Ns + Sham group; xxxP < 0.001 when compared to Losartan + Sham group)
not change the pattern of the corticosterone response to FSS in the male rats compared to the NS + Stress rats, which were exposed to FSS during the experiments. The two-way ANOVA analysis showed no significant interaction or significant main effect of losartan on serum concentration of corticosterone.

Discussion

The concept of this study was based on the different impacts of losartan on anxiety responses, cognitive processes, and pain. While this matter has already been discussed from various points of view, interpretation of different aspects of this drug’s action is difficult if not impossible. Therefore, we highlight our findings on this controversial notion in the following section.

In the present study, we examined the effect of losartan on FSS and also the changes that losartan caused in the motor and anxiety-like behavior. Anxiety states were assessed with open field test, which is a standard behavioral model in rodents (28). Our finding indicated that losartan is involved in decreasing anxious behaviors and prevented stress responses that are caused by FSS. This test gives a valuable measure in the understanding of anxious behavior and locomotor activity. Center/perimeter area expresses an alteration of preference, from the less dangerous or exposed position (perimeter areas) to the higher risk possibility (center areas). This parameter is a manifestation of the anxiolytic effect of stressed rats. An increase in central area duration is a parameter classically linked to locomotor activity (29). The reduction found in the time spent in the center in NS + stress compared to NS + sham strongly indicates the increasing anxiety in this group. In contrast, Losartan + stress group increased central time compared to NS + stress group. This is in line with previous findings, showing that losartan reduces the anxious behaviors caused by stress (30). In rodents, an increase in fear response has been associated with impairment in exploratory and locomotor activities through heightening anxiety-like behavior such as increase in the frequency of defecation, and higher grooming and rearing frequency (29).

Hyperactivity of NS + stress animals was shown by more tendencies towards perimeter areas of the open field compared to NS + sham. Interestingly, mobility alternations were consistent with the previous parameter and showed motor hyperactivity in rats which normal saline and stress induced. Meanwhile, losartan did not affect total distance moved in sham and losartan groups compared to the saline-injected group. The velocity followed the same pattern and was not affected in any of the experimental conditions. Another parameter was the grooming behavior, characterized as a response associated with the stressful situation. Therefore, the animals under stress would spend more time in grooming than the animals in the control condition (31). This could explain our findings with stress that significantly increased the grooming behavior. However, it seems losartan injection had no significant effect on the grooming behavior induced by stress. It was suggested that stress induces activities that represent a diminished motivation to interact with the environment, which would explain the decreased exploratory behavior after FSS in our study. This is also supported by the finding that stressed rats increased grooming behavior, which is considered a behavioral response that follows alterations provoked by anxiogenic stimuli (4). However, the results of the present study showed that the corticosterone levels after the FSS assay did change and this finding was in line with a study that was conducted by Takeuchi et al (32). The acute stress-induced glucocorticoid increase is usually a beneficial response that helps the body avoid injury. Although the plasma corticosterone level on the day after stress session tended to decrease in the Losartan + Stress group, two-way ANOVA didn’t show any significant changes by Angiotensin II type 1 receptor blocker.

The losartan treatment of stress induced in the present study can be explained by previous data showing that chronic blockade of the AT 1 receptor within amygdala improved anxiety responses (33). Moreover, recent clinical uses give additional support to the idea that AT1 receptor inhibitors could potentially be used as anxiolytic drugs. Thus, Administration of losartan which is a selective AT1 receptor antagonist might reduce motor hyperactivity and anxiogenic behavior in stressed rats via inhibition of AT 1 receptor in the amygdala (29).

Increasing evidence suggests that losartan plays a role not only in the reduction of anxiety behavior but also in learning and memory (31). In agreement with these reports, the present work showed that injection of losartan significantly improved learning and memory, examined using the passive and active avoidance tests. In the shuttle box test, losartan significantly increased the step-through latency during the retention test (memory), while the injection of normal saline did not show a significant effect (9).

The present results showed that losartan improved memory function. It can be concluded that losartan has a positive effect not only on blood pressure but also on memory function. The possible mechanism would be the involvement of brain angiotensin II (AII) in cognitive processes, including learning and memory (34). The hippocampus is a key brain structure in memory formation. Previous studies indicated that administered losartan (an antagonist of the Ang II type 1 receptors) suppresses the impaired effects in the rat hippocampus. The expression of Ang II is high in the hippocampus, therefore its role in the processing of cognitive functions, such as the hippocampus is undeniable. Furthermore, the recent research on the role of AT2 receptors in cognitive processes provided evidence about their positive effect on cognitive processes (9).

An interesting area within the context of stress-pain interactions is the relationship between chronic pain and affective disorders. As mentioned before exposure to stressful conditions, results in SIH (10). Following the effect of losartan treatment on physical and psychological stressors, it significantly changed the pain threshold in the tail flick test. Also, in agreement with current findings, some investigations showed the same results that losartan diminished pain (15, 16). Thus, in the present study, we determined that the administration of losartan may produce an antinociceptive effect.

Conclusion

It seems losartan has positive impacts on anxiety,
memory, and pain. Although further studies are needed to determine whether using a different dosage of losartan leads to the same outcomes.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Shoji H, Mizoguchi K. Brain region-specific reduction in c-Fos expression associated with an anxiolytic effect of yokukansan in rats. J Ethnopharmacol. 2013;149:93-102.

2. Leiby-Panissi CRA, Ferrarese AA, Terzian ALB, Menescal-de-Oliveira L. Serotonergic activation of the basolateral amygdala and modulation of tonic immobility in guinea pig. Brain Res Bull. 2006;69:356-364.

3. Li S, Wang C, Wang W, Dong H, Hou P, Tang Y. Chronic mild stress impairs cognition in mice: from brain homeostasis to behavior. Life Sci. 2008;82:934-942.

4. Leite-Panissi CRA, Ferrarese AA, Terzian ALB, Menescal-de-Oliveira L. Serotonergic activation of the basolateral amygdala and modulation of tonic immobility in guinea pig. Brain Res Bull. 2006;69:356-364.

5. Mesfin M, Asres K, Shibeshi W. Evaluation of anxiolytic activity of the essential oil of the aerial part of Foeniculum vulgare Miller in mice. BMC Complement Altern Med. 2014;14:310-316.

6. Simoons V, Istok E, Hytten S, Hirvonen A, Natänen R, Tervaniemi M. Psychosocial stress attenuates general sound processing and duration change detection. Psychophysiology. 2007;44:30-38.

7. Pettit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. 2005;177:245-255.

8. Maeng LY, Shors TJ. The stressed female brain: neuronal activity in the prelimbic but not infralimbic region of the medial prefrontal cortex suppresses learning after acute stress. Front Neural Circuits. 2013;7:198.

9. Ongali B, Nicolakakis N, Tong X-K, Aboulkassim T, Papadopoulos P, Rosa-Neto P, et al. Angiotensin II type 1 receptor blocker losartan prevents and rescues cerebrovascular, neuropathological and cognitive deficits in an Alzheimer’s disease model. Neurobiol Dis. 2014;68:126-136.

10. Jennings EM, Okine BN, Roche M, Finn DP. Stress-induced hyperalgesia. Prog Neurobiol. 2014;122:1-18.

11. Aghaei I, Khedr MM, Nazeri M, Karrer SA. The involvement of KATP channels in morphine-induced antinociception and hepatic oxidative stress in acute and inflammatory pain in rats. Fundam Clin Pharmacol. 2013;27:623-631.

12. Kalra J, Chaturvedi A, Kalra S, Chaturvedi H, Dhasmana D. Modulation of pain perception by ramipril and losartan in human volunteers. Indian J Physiol Pharmacol. 2008;52:91-96.

13. Miguez G, Laborda MA, Miller RR. Classical conditioning and pain: Conditioned analgesia and hyperalgesia. Acta Psychol. 2014;145:10-20.

14. Quintero L, Cardenas R, Suarez-Roca H. Stress-induced hyperalgesia is associated with a reduced and delayed GABAergic inhibition control that enhances post-synaptic NMDA receptor activation in the spinal cord. Pain. 2011;152:1909-1922.

15. Nemoto W, Ogata Y, Nakagawasai O, Yaoita F, Tanado T, Tan-No K. Intraarticular administration of losartan, an AT1 receptor antagonist, produces an antinociceptive effect through the inhibition of p38 MAPK phosphorylation in the mouse formalin test. Neurosci Lett. 2015;585:17-22.

16. Nemoto W, Nakagawasai O, Yaoita F, Kanno S-I, Yomogida S, Ishikawa M, et al. Angiotensin II produces nociceptive behavior through spinal AT1 receptor-mediated p38 mitogen-activated protein kinase activation in mice. Mol Pain. 2013;9:1.

17. Mohammad-Zadeh M, Azhdar-Zarmehri H, Mosafi F, Haghdooest-Yazdi H, Nazeri M, Shabani M. Modulation of different phases of formalin test by force swim stress. Basic Clin Neurosci. 2014;5:303-307.

18. Aghaei I, Shabani M, Doustar N, Nazeri M, Dephour A. Peroxisome proliferator-activated receptor-γ activation attenuates mouse and cognition impairments induced by bile duct ligation in a rat model of hepatic cirrhosis. Pharmacol Biochem Behav. 2014;1:20:133-139.

19. Parsania S, Shabani M, Moazzami K, Razavinasab M, Larizadeh MH, Nazeri M, et al. Gender difference in motor impairments induced by chronic administration of vinblastine. Iran J Basic Med Sci. 2014;17:433-440.

20. Razavinasab M, Shamsizadeh A, Shabani M, Nazeri M, Allahtavakoli M, Asadi-Shekaari M, et al. Pharmacological blockade of TRPV1 receptors modulates the effects of 6-OHDA on motor and cognitive functions in a rat model of Parkinson’s disease. Fundam Clin Pharmacol. 2013;27:632-640.

21. Shabani M, Larizadeh MH, Parsania S, Hajali V, Shojaei A. Evaluation of destructive effects of exposure to cisplatin during developmental stage: no profound evidence for sex differences in impaired motor and memory performance. Int J Neurosci. 2012;122:439-448.

22. Nazeri M, Shabani M, Larizadeh MH, Golchin L, Razavinasab M, Abareghi F, et al. Simultaneous impairment of passive avoidance learning and nociception in rats following chronic swim stress. Adv Biomed Res. 2015:9:93.

23. Golchin L, Shabani M, Harandi S, Razavinasab M. Pistachio supplementation attenuates motor and cognition impairments induced by cisplatin or vincristine in rats. Adv Biomed Res. 2015;4:92.

24. Meymandi MS, Sepehri G, Abdolsamadi M, Shabani M, Heravi G, Yazdanpanah O, et al. The effects of co-administration of pregabaline and vitamin E on neuropathic pain induced by partial sciatic nerve ligation in male rats. Inflammopharmacology. 2017;25:237-246.

25. Shabani M, Nazeri M, Parsania S, Razavinasab M, Zangibadi N, Esmaeilpour K, et al. Walnut consumption protects rats against cisplatin-induced neurotoxicity. Neurotoxicology. 2012;33:1314-1321.

26. Abbassian H, Esmaeili P, Tahamtan M, Aghaei I, Vaziri Z, Shebani V, et al. Cannabinoid receptor agonism suppresses tremor, cognition disturbances and anxiety-like behaviors in a rat model of essential tremor. Physiol Behav. 2016;164:314-320.

27. Aghaei I, Arjmand S, Yousefzadeh Chabok S, Tondar R. Neuroprotective mechanism of losartan and its potential agonist activity on AT1 receptor. Basic Clin Neurosci. 2015;28:420-427.

28. Workman JL, Fonken LK, Gusfa J, Kassouf KM, Nelson RJ. Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. Pharmacol Biochem Behav. 2011;100:25-32.

29. Lázaro-Lozada L, Caif F, García S, Fraile M, Landa AI, Baiardi G, et al. Cannabinoid receptor agonism suppresses tremor, cognition disturbances and anxiety-like behaviors in a rat model of essential tremor. Physiol Behav. 2016;164:314-320.

30. Kumar A, Singh B, Mishra J, Sah SP, Pottabathini RJ. Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. Pharmacol Biochem Behav. 2011;100:25-32.

31. Miranda-Melo MD, Pérez PA, Gargiulo PA, Casarsa BS, Bregnonzi C, Biaardi G. Fear-Potentiated behaviour is
modulated by central amygdala angiotensin II receptors stimulation. Biomed Res Int. 2014;183248: 1-7.
32. Takeuchi T, Matsunaga K, Sugiyama A. Antidepressant-like effect of milk-derived lactoferrin in the repeated forced-swim stress mouse model. J Vet Med Sci 2017; 10;79:1803-1806.
33. Pechlivanova DM, Stoynev AG, Tchekalarova JD. The effects of chronic losartan pretreatment on restraint stress-induced changes in motor activity, nociception and pentylentetrazol generalized seizures in rats. Folia med. 2011;53:69-73.
34. Fogari R, Mugellini A, Zoppi A, Derosa G, Pasotti C, Fogari E, et al. Influence of losartan and atenolol on memory function in very elderly hypertensive patients. J Hum Hypertens. 2003;17:781-785.