

Effects of neonatal capsaicin treatment on stress induced analgesia and hyperalgesia in Tail-Flick test in male rats

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Abstract

Background & Objective: It is reported that acute forced swimming stress induces analgesia immediately, and chronic stress induces hyperalgesia. Whereas in response to nociceptive stimulation, small-diameter C-fibers of the excitatory system in the dorsal horn of the spinal cord are activated, therefore, in the present study, the effects of C-fiber lesion in stress and dexamethasone-induced analgesia and hyperalgesia in acute and chronic forms were investigated using Tail-Flick test. **Methods:** Adults Wistar male rats (180-200 g) were assigned into three groups (n=7): C-normal (intact C-fibers), sham (received capsaicin vehicle at neonate stage) and C-lesion (received capsaicin at neonate stage). Forced swim stress (10 min/day) in water (18±1 °C) was considered as acute stress and repeated daily forced swim stress as chronic stress, also single-dose of dexamethasone (2 mg/kg, i.p.) was considered as acute dexamethasone and repeated for three days as chronic dexamethasone. Neonatal capsaicin treatment was used for C-fibers depletion. The nociceptive thermal threshold was assessed using Tail-Flick test. **Results:** In C-lesion group, thermal pain sensitivity was reduced ($P<0.001$). Acute stress in C-normal group, reduced pain ($P<0.001$) and in C-lesion group, it caused deeper antinociception in Tail-Flick ($P<0.001$). Chronic stress and acute-chronic dexamethasone in C-normal group, created hyperalgesia ($P<0.001$) and induced analgesia in C-lesion groups ($P<0.01$).

Conclusion: It seems that presence of C-fiber is so important in thermal pain transmission in Tail-Flick test; therefore, C-fiber lesion, reduces pain sensitivity (analgesia), increases antinociception effects of acute stress, decreases hyperalgesia of chronic-stress and acute-chronic dexamethasone.

INTRODUCTION

Noxious stimuli are carried in the central nervous system (CNS) by two kinds of fibers; anatomically, there are two broad groups of sensory fibers: myelinated A-fibers and unmyelinated C-fibers with the smaller diameter.^{1,2} Peripheral terminals of A -fibers are mechanical receptors that usually, do not respond to thermal and chemical stimuli whilst C nociceptors respond to mechanical, chemical and thermal stimuli.³ TRPV-1 is a ligand-gated, non-selective cation channel. In addition to being sensitive to capsaicin, TRPV-1 responds to thermal stimuli, protons and its activity might be enhanced within the acidic environment of inflamed tissues. In addition of C-fibres also, there are existed on some A δ -fibers.⁴⁻⁶ Pain due to A -fibers stimulation travels quicker (5-30 meters/second) than the C-fibers (0.5-2 meters/second).⁷

Analgesia or hyperalgesia occurs in animals under different conditions such as stress.¹ An increase in pain threshold following acute exposure to painful or stressful events was demonstrated and introduced as stress-induced analgesia.^{8,9} Although it has been shown that chronic forced swimming stress also induces hyperalgesia. On the other hand, acute administration of dexamethasone (2 mg/kg, i.p.) produces hyperalgesia almost after 30 minutes in mimicry of corticosterone secretion.¹⁰ Nevertheless of the existence of remarkable information on stress-induced hyperalgesia, what remains to be fully elucidated is the involvement of different systems or paths in this circumstances.¹

Therefore, the roles of C-fibers during the effects of stress on pain are questioned in this study. Dexamethasone as a synthetic glucocorticoid can

help for mimicry of a part of the stress activation effects on pain and is used here for more elucidation of the roles of C-fibers on the pain system while of stress. Whereas importance of C-fibers on stress-induced analgesia/hyperalgesia and dexamethasone-induced hyperalgesia are not elucidated in detail; this research is done to clarify them at least partially.

METHODS

Animals

Experiments were carried out on male Wistar rats weighing 180–200 g. The animals were housed in standard laboratory conditions (12 h lighting cycle and 22 ± 1 °C temperature) with free access to food (chow pellets) and tap water. All of the procedures were performed in accordance with guidelines for the care and use of laboratory animals.¹¹ Animals were divided into three groups (n=7); C-normal (intact C-fibers), sham (received capsaicin vehicle at neonate stage) and C-lesion (received capsaicin at neonate stage). One session of forced swim stress (10 min/day) in water (18 ± 1 °C) was considered as acute stress. Repeated daily forced swim stress (three sessions) was considered as chronic stress, single-dose of dexamethasone (2 mg/kg, i.p.) was considered as acute treatment, and a three successive daily injections of dexamethasone was considered as chronic treatment.

Capsaicin preparation

Capsaicin powder (Sigma) was dissolved in a solvent consisting of ethanol, Tween 80 and saline in a ratio of 1:1:8 respectively to prepare a 0.5% solution of capsaicin. Neonatal treatment of rats with capsaicin (50 mg/kg) within 48h after birth effectively destroys C-fibers.¹²⁻¹⁴ Therefore; capsaicin (50 mg/kg, i.p.) was injected into the first postnatal day¹⁵ then rat neonates were allowed to grow up until adulthood. Efficacy of capsaicin treatment in depleting C-fibers was also assessed by corneal chemosensitivity test.¹⁶ Corneal chemosensitivity is principally mediated by C-fibers¹⁴ and its significant reduction, as in our experiment is happened, means significant depletion of C-fibers.^{17,18} In this study, those capsaicin-treated animals were considered as C-lesion groups which the number of wipe against the administration of one drop of 1% ammonium hydroxide in their right eye was reduced during the first 10 seconds.

Forced swim stress

In order to prevent the data changing due to the circadian rhythm, especially what related to hypothalamic–pituitary–adrenal (HPA) axis activity, all tests were performed between 10 to 14 o'clock. Rats were subjected to a forced swim procedure (10 min/day) in a cylindrical plastic container (diameter=35 cm, height=50 cm) which was filled with water at temperature of 18 ± 1 °C with a depth of 40 cm. After the swimming sessions, rats were immediately dried using a towel^{19,20} and then subsequent assessments were done.

Tail-Flick test

Analgesic responses were measured with the Tail-Flick (TF) test.²¹ To measure the latency of the TF response; mice were gently held with the tail and put on the apparatus (Sparco, Iran). The TF response was elicited by applying radiant heat to the middle 1/3 of the tail. The intensity of the heat stimulus was set to provide a predrug TF response time of 4 to 6 sec. The inhibition of the TF response was expressed as percent of maximal possible effect (MPE%), which was calculated as $[(T1-T0)/(T2-T0)]\times 100$, where T0 and T1 are standing for the TF latencies before and after stress or injection of saline (1mg/kg, i.p.) or dexamethasone (2 mg/kg, i.p.)¹⁰, and T2 is standing as cut-off time, which was set at 15 sec for prevention of any possible tissue damage.²²

Experimental Protocols

Tail-Flick latencies were measured three times before injection of (acute/chronic) dexamethasone (2 mg/kg, i.p.) or saline (drug solutions) (1 ml/kg, i.p.) and/or swimming stress then their mean considered as T0. In acute mode, 30 minutes after injection of dexamethasone or saline and immediately after swimming stress latency of the TF response were measured for three times then their mean considered as T1 and in the chronic mode, T1 calculated at fourth day-after three days administrations of dexamethasone or stress. Because the mentioned formula calculates the percent of maximal possible effect regard to the analgesia, sometimes MPE% could be calculated negative, and it means hyperalgesia, to have the percent of maximal possible effect in the aspect of hyperalgesia, a normalizing correction coefficient is necessary, because hyperalgesia is a reduction of pain threshold or tail flick latency under the pain threshold base line before drug

administration just between the time ranges of zero to base line. Thus, for the cases of hyperalgesia the correction coefficient formula would be: (cut-off time - baseline latency / baseline latency - 0) which it has to multiply by MPE%, which already calculated for each case.

Statistical analysis

Results are expressed as mean±SEM. One-way ANOVA followed by Tukey's test were used for statistical analysis. P-values less than 0.05 were considered as statistically significant.

RESULTS

In contrast to control and sham (C-normal) animals results for the test of chemosensitivity of the cornea before any other tests had shown that after dropping of the ammonium hydroxide within the eye, wipes were diminished in infant capsaicin treated animal ($P<0.001$) (Figure 1). These results provided a confirmation for C-fiber elimination happening in infant capsaicin treated (C-lesion) animals.

However the effects of infant capsaicin treatment for C-fiber lesioning on thermal pain sensation was needed to be clarify in the mature animal using TF test, this results making it possible to interpret the effects of C-fiber lesioning on the acute and chronic stress and dexamethasone effects on thermal pain threshold.

Effects of presence/deficit of C-fibers on pain threshold in Tail-Flick test

In C-normal animals, administration of saline (drug solvents) could not change the latency of the TF response. This result for sham animals was the same. Comparison between the groups has shown as percent of MPE in the Figure 2. As it is shown, the difference between C-normal and sham animals were not significant but after C-fiber depletion, a significant analgesia occurred in contrast to C-normal animals.

Effects of acute stress on pain in presence/deficit of C-fibers in Tail-Flick test

Immediately, after the acute forced swimming stress in the C-normal animals, TF responses showed an increase in the pain threshold ($P<0.001$). In C-lesion animals, acute stress had the same effect, but the percent of MPE in C-lesion animals after acute stress was more than of the C-normal animals ($P<0.001$); therefore, in C-lesion animal stress induced analgesia was more potent, so it is possible that in C-lesion animals, C-fibers depletion has decreased pain sensitivity (analgesia) and increased antinociception effects of acute stress ($P<0.001$) (Figure 3).

Effects of chronic stress on pain in presence/deficit of C-fibers in Tail-Flick test

Latency of the TF responses in C-normal group, after repeated daily forced swim stress (three

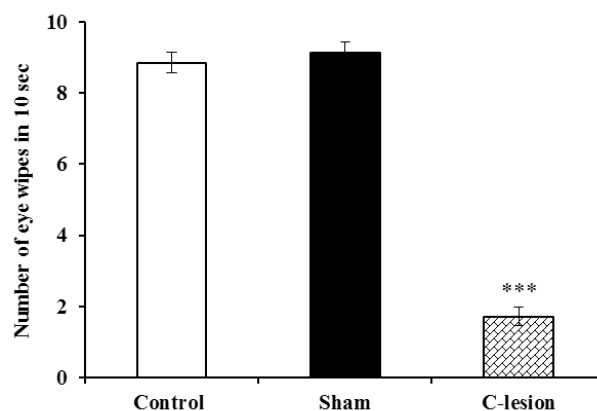


Figure 1. Eye wipe comparison between control, infant capsaicin vehicle treated (sham), and infant capsaicin treated animals (C-lesion) after dropping of ammonium hydroxide in the eye. In group of infant capsaicin treating, eye wipes number is diminished in contrast to control and sham, so C-normal is considered for control and sham groups animals and C-lesion for animals of group of infant capsaicin treating. Data are shown as mean±SEM (***) $P<0.001$ in contrast to control and sham) ($n=7$).

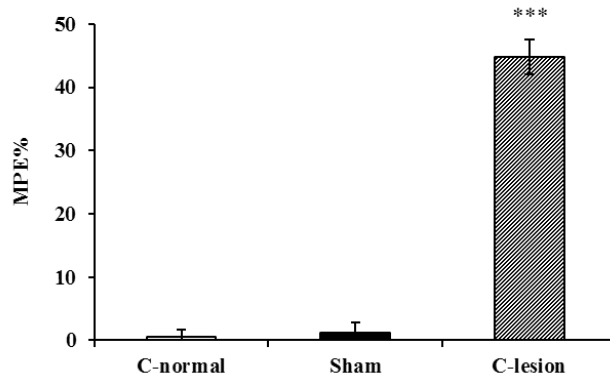


Figure 2. Comparison of presence/deficit C-fibers effect on pain in Tail-Flick test. In C-lesion group analgesia was increased (***) $P<0.001$ compares to C-normal group). Results represent as mean \pm SEM (n=7 in each group).

session), in the fourth day was shorter than its base line that is indicated as hyperalgesia ($P<0.01$). But interestingly in C-lesion animal there was a reduction of pain threshold or analgesia after Chronic stress, unlike the hyperalgesic effects induced by chronic swimming stress in C-normal group ($P<0.01$) (Figure 4).

Effects of acute dexamethasone on pain in presence/deficit of C-fibers in Tail-Flick test

In C-lesion group, there was a pain threshold elevation or analgesia in contrast to C-normal animals while acute dexamethasone (2 mg/kg, i.p.) in C-normal group showed hyperalgesia ($P<0.001$) (Figure 5). Figure 5 also shows that,

this hyperalgesic effect of dexamethasone was attenuated while dexamethasone administrated to C-lesion animals ($P<0.01$). However, acute dexamethasone application in C-lesion group reduced analgesia that induced by C-fiber depletion ($P<0.001$) (Figure 5).

Effects of chronic dexamethasone on pain in presence/deficit of C-fibers in Tail-Flick test

Chronic dexamethasone administration reduced pain threshold in C-normal group ($P<0.001$). Analgesic effect of C-fibers depletion has changed to hyperalgesia after chronic dexamethasone administration ($P<0.001$), however the intensity of hyperalgesia induction was lesser than the

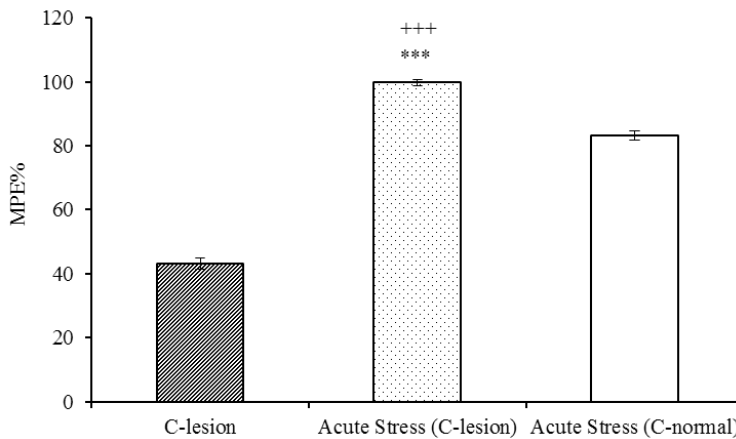


Figure 3. Effect of acute stress on pain in presence/deficit C-fibers in Tail-Flick test. Acute stress increased analgesia in C-lesion group (***) $P<0.001$ compares to C-normal group), and acute stress aggravated C-fibers depletion induced analgesia (+++) $P<0.001$ compares to no stress in C-lesion group). Results represent as mean \pm SEM (n=7 in each group).

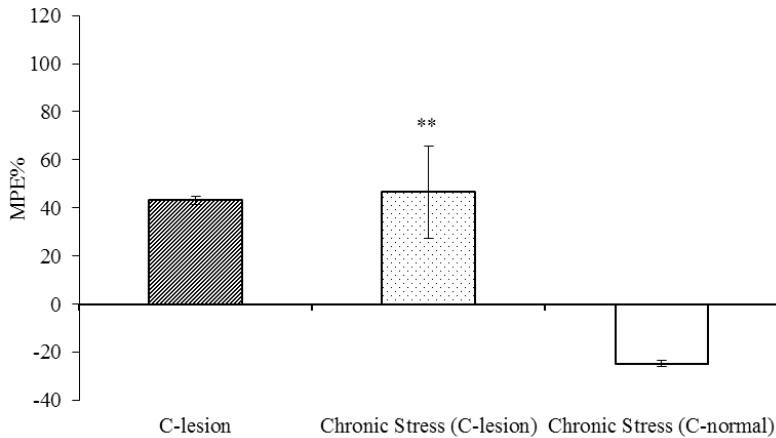


Figure 4. Effect of chronic stress on pain in presence/deficit C-fibers in Tail-Flick test. Chronic stress reduced hyperalgesia in C-lesion group (** $P < 0.01$ compares to C-normal group), and chronic stress had no effect on C-fibers depletion induced analgesia. Results represent mean \pm SEM, (n=7 in each group).

hyperalgesia induction in C-normal animals ($P < 0.001$) (Figure 6).

DISCUSSION

In the present study, we used Capsaicin, the pungent ingredient of red peppers, for C-fiber depletion. Our data showed a reduced thermal sensitivity after C-fiber depletion. Acute stress in C-normal animals, enhanced pain threshold in Tail-Flick test, it is probable that, this analgesia was induced via C-fibers. As it is known, stress activates neural systems that inhibit pain sensation. This adaptive response, referred to stress-induced

analgesia (SIA), depends on the recruitment of brain pathways.²³ Although endogenous opioid peptides are known as key functions in this process^{24,25}, but another neurotransmitters are also known to be involved in SIA, including endocannabinoids.²³ Cannabinoids can suppress the noxious stimulus-evoked neuronal activity in nociceptive neurons in the spinal cord and thalamus. Different modalities of noxious stimulation (mechanical, thermal, chemical), are modulated by cannabinoid receptors, and correlate with the antinociceptive effects of cannabinoids. Two subtypes of cannabinoid receptors CB1 and CB2 have been identified. CB1 is expressed

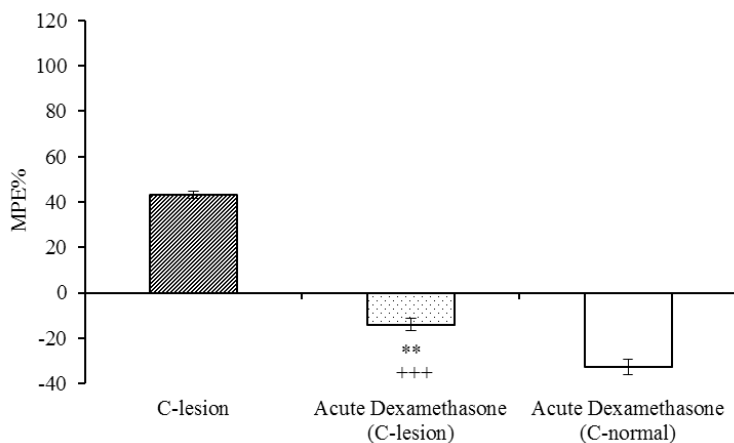


Figure 5. Effect of acute dexamethasone application on pain in presence/deficit C-fibers in Tail-Flick test. Acute dexamethasone application in C-lesion group attenuated hyperalgesia (** $P < 0.01$ compares to C-normal group), and acute dexamethasone application reduced C-fibers depletion induced analgesia (+++ $P < 0.001$ compares to C-lesion group). Results represent mean \pm SEM (n=7 in each group).

on central and peripheral neurons. Activation of the CB1 receptor is negatively coupled to adenylate cyclase and blocks excitability and activation of primary afferents.²⁶ Cannabinoids also suppress C-fiber-evoked responses in spinal dorsal horn neurons. One possible mechanism for the antihyperalgesic actions of cannabinoids is suggested by cannabinoid-induced suppression of windup and noxious stimulus-induced central sensitization that is done by both central and peripheral sites.²⁷ Results showed acute stress in C-lesion groups caused deeper antinociception in Tail-Flick than C-normal groups that in comparison with C-lesion groups these antinociception effects had augmented too. Probably acute stress did some part of its antinociception effects by C-fibers and some of them via A -fibers because in addition of C-fibers reduction- induced analgesia; acute stress produces deeper antinociception, so it can be possible that cannabinoids act on remained a few of C-fibers or do it likely by stimulation of cannabinoid receptor on A -fibers. Therefore, investigation of cannabinoid receptor on A -fibers is also suggested. In C-normal animals, chronic stress induced hyperalgesia; chronic stress can have deep effects on 5-hydroxytryptamine (5HT) system. 5HT pathways determine levels of pain by modulating nociceptive responses.²⁸ 5HT acting at multiple receptors exerts a complex long-recognized control of pain mechanisms through descending pathways.^{29,30} 5-HT3 receptors (5HT3R) are also expressed within the central nervous system in limbic structures, brain stem,

and spinal cord. At the dorsal horn level, they are localized presynaptically in the superficial laminae³¹ and also 5HT3R expresses in primary afferent fibers. Activation of 5HT3R on wide dynamic range (WDR) neurons leads to depolarization and renders *N*-methyl-d-aspartate (NMDA) receptors capable of activation by glutamate. 5HT3 receptor antagonists (5HT3RA) have been shown to reduce the nociceptive responses in some dorsal horn neurons and primary nociceptive afferent fibers.^{29,30} Therefore, we can probably suppose that chronic stress can exert its hyperalgesic effect via C-fibers and 5HT3 receptors at least partially in Tail-Flick test. Therefore reduction of hyperalgesic effect of chronic stress in C-lesion animals could be probably parallel with this assumption.

Both acute and chronic administrations of dexamethasone in C-normal animals reduced thermal pain threshold in Tail-Flick test. Central receptors for glucocorticoids (GCs) and NMDA interact and play a significant role in pain after peripheral nerve injury.^{32,33} This is happen possibly by initiating calcium influx and activation of protein kinases that in turn facilitate NMDA receptor up-regulation.³⁴ Central GC receptors are located in the dorsal horns of the spinal cord and are up-regulated during peripheral nerve injury. Since NMDA receptors are enhanced by GCs, the timing of GC treatment can explain the paradoxical outcome with treatment. Although GCs are expected to have anti-inflammatory effects at the site of tissue injury, up-regulation of GC

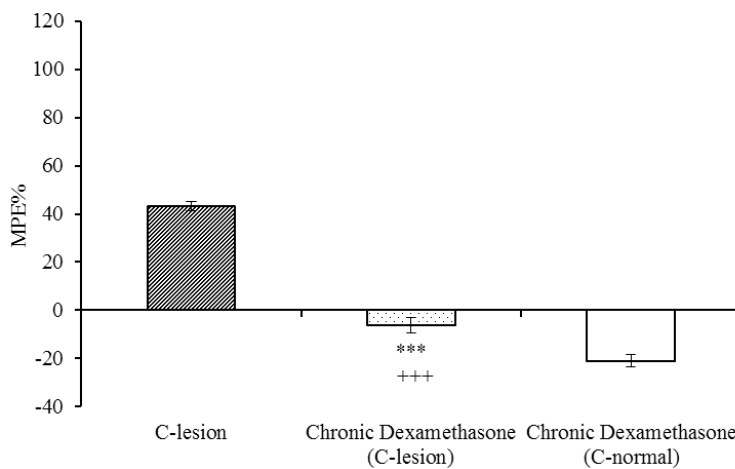


Figure 6. Effect of chronic dexamethasone on pain in presence/deficit C-fibers in Tail-Flick test. Chronic dexamethasone application in C-lesion group attenuated hyperalgesia (***)P<0.001 compare to C-normal group), and chronic dexamethasone application reduced C-fibers depletion induced analgesia (+++)P<0.001). Results represent mean±SEM, (n=7 in each group).

receptors in the spinal cord could enhance NMDA receptors, leading to facilitated pain transmission³⁵ and induce hyperalgesia. C-fibers depletion in C-lesion animals diminished the hyperalgesic effects of chronic-acute dexamethasone treatment. It seems that the presence of C-fibers is so important in thermal pain transmission during (acute-chronic) dexamethasone treatment in Tail-Flick test as it could be the same in acute-chronic stress experimental status. Whereas decreasing the number of C-fibers attenuates hyperalgesia induced by chronic stress and acute-chronic dexamethasone, thermal and stimulant intensity to create hyperalgesia same as what happened in C-normal might be further than normal. Therefore the remained faster A -fibers⁷ after C-fiber depletion probably conveys thermal pain quicker but with less sensitivity, because in complete presence of C-fibers, thermal threshold is diminished or hyperalgesia is produced by chronic stress and acute-chronic dexamethasone treatment with a higher efficacy than C-lesion animals.

Therefore, according to the results of this study, it can be concluded that C-fiber lesioning, reduces pain sensitivity and produces analgesia, also increases analgesic effect of acute stress and decreases hyperalgesic effects of chronic-stress and acute-chronic dexamethasone.

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DISCLOSURE

Conflict of interest: None

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