Title: Effect of Acute Administration of Caffeine and the Role of Nitric Ox-ide Pathway in an Animal Model of Chronic Constriction Injury of the Sciatic Nerve

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Abstract

Background: Partial peripheral nerve injury often results in chronic pain, including hyperalgesia and allodynia. Caffeine as a non-selective antagonist of adenosine receptors (ARs) has protective effects on neuropathic pain. Since, in the central effects of caffeine nitric oxide (NO) partially is involved, we therefore investigated the effects of acute caffeine administration on neuropathic pain, focusing on A1 and A2 receptors and the possible role of NO.

Methods: Following chronic constriction injury (CCI), male Wistar rats were administrated with caffeine (10, 50 and 100mg/kg). Also, groups of animals received L-NAME (30mg/kg) or L-arginine (100mg/kg) either alone or as before treatment with 50 mg/kg of caffeine. Rats were tested for hyperalgesia and allodynia at 4, 7, 14, 21 and 28 days following CCI.

Results: Administration of 10 mg/kg of caffeine significantly increased cold allodynia, while 50 and 100 mg/kg of caffeine, decreased mechanical allodynia and thermal hyperalgesia. Pre-treatment with L-NAME before caffeine administration decreased cold and mechanical allodynia, and thermal hyperalgesia. Treatment with L-arginine before caffeine administration, increased thermal hyperalgesia and decreased cold allodynia.

Conclusions: The present data show that caffeine dose-dependently affects the pro-analgesic or anti-analgesic states in the CCI model.

Keywords: Neuropathic Pain, Caffeine, L-NAME, L-Arginine, Nitric Oxide, Adenosine receptors

Introduction

Neuropathic pain (NP) is a chronic disease that results from damage to the peripheral and central nervous system (Jensen et al., 2011). It can cause complex changes in the cognitive and emotional functions (Jensen et al., 2011). NP is characterized by the presence of allodynia, which is pain caused by an innocuous stimulus, and hyperalgesia, which is an exaggerated response to a painful stimulus (Treede et al., 2008). Tumors, metabolic disorders, viral infections, and injury to the peripheral or central nervous system are considered causes of NP. (Cervero, Laird, & García-Nicas, 2003). The main drug treatments for NP include opioids, anticonvulsants, antidepressants and topical agents. (Attal, 2012). However, treatments have adverse side effects including tolerance and physical dependence without complete pain relief. (Grzanna, Lindmark, & Frondoza, 2005). Therefore, further pharmacological interventions are necessary to relieve these side effects.

Caffeine is related to the purine alkaloid family and is the main active constituent in tea, coffee and energy drinks (Nieber, 2017). Due to its mild stimulant effect, caffeine is the most widely consumed psychoactive substance in western countries (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Evidence suggests that some doses of caffeine have antinociceptive effects (Person et al., 1985; Wu, Hao, Fredholm, Wiesenfeld-Hallin, & Xu, 2006). Also, one of non selective antagonist of adenosine receptors (ARs) is caffeine (Tchekalarova, Kubová, & Mareš, 2010). Adenosine has four G protein-coupled receptors A2B, A1, A2A, and A3 (McGaraughty, Cowart, Jarvis, & Berman, 2005). The antinociceptive effect of caffeine is probably due to antagonism of the A_{2A} receptors (Wu et al., 2006; Zhang, 2001). Nitric oxide (NO) is synthetized from L-arginine by different isoforms of nitric oxide synthase (NOS)(Akula, Dhir, & Kulkarni, 2008). NOS displays three isoforms including endothelial, neuronal, and inducible isoforms (Garthwaite, 2008). Finding suggests that NO plays a complex role in pain modulation (Basbaum, Bautista, Scherrer, & Julius, 2009; Schaible & Richter, 2004). On the other hand, it

is known that caffeine can modulate NO production (Kayir & Uzbay, 2004; López-Muñoz, Castañeda-Hernández, & Granados-Soto, 1996). Whereas activation of the A_{2A} receptors stimulates NO production activation of the A_1 receptor decreases NO production. It is suggested that effects of caffeine on NO synthesis may be due to antagonism of the ARs (Bruce, Yates, & Thomas, 2002). Despite, it seems that the action mechanism of caffeine is not only through antagonism with ARs and some other mechanisms like the NO-cGMP pathway can be involved. (Kayir & Uzbay, 2004; Orrú et al., 2013). Therefore, the aim of our study was to indicate the effects of acute administration of caffeine on NP threshold. The rule of the NO pathway was also considered.

Materials and methods

Animals

Eighty eight male Wistar rats weighting 220–250 g were used in this study. Three or four rats were placed in per cage in a 12 h light/dark cycle in a temperature- (22±2 °C) and humidity-controlled (55±5 %). Water and food and were available *ad libitum*. All experiments were carried out in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by Ethical Committee of Kashan University of Medical Sciences (ethical code: I.R.KAUMS.MEDNT.REC.1397.040).

Drugs

Caffeine, L-NAME and L-arginine were purchased from Sigma-Aldrich Company. The drugs were diluted with normal saline and administered intraperitoneally.

Neuropathic pain model

We used the animal model of chronic constriction injury (CCI) as described previously (Bennett & Xie, 1988). The subjects were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (50 mg/kg) (Verdi, Jafari-Sabet, Mokhtari, Mesdaghinia, & Banafshe, 2013). The common portion of the left sciatic nerve was exposed and separated from the adjacent connective tissue. Four ligatures (4-0 intestinal chrome) were loosely tied around the nerve, so as not to interrupt blood circulation through the superficial epineural vessels. After surgery, every rat was separately placed in cage (Verdi et al., 2013).

Animal groups

The study protocol included 11 groups of rats comprising of 8 animals in each group. Three control groups were entered the study. One group served as age matched non-ligation control (CON). Two control groups of rats were subjected to the sciatic nerve ligation with (CCI+Saline) or without (CCI) saline administration. Sham group (sham) experienced similar surgery, except that the left sciatic nerve was exposed with no ligation. Three groups of CCI animals received 10 (Caf.10), 50 (Caf.50) and 100 (Caf.100) mg/Kg caffeine, Two groups of rats were administered by 100 mg/kg L-arginine (L-ARG) and 30 mg/kg L-NAME (L-NAME). Also two groups of animals were pre-treated by 30 mg/kg L-NAME (L-NAME+Caf) and 100 mg/kg L-arginine (L-ARG+Caf) 30 minutes before administration of the effective dose of caffeine (50 mg/kg) (Pottabathini, Kumar, Bhatnagar, & Garg, 2015).

The same groups of animals were introduced to all tests.

Allodynia and hyperalgesia

The mechanical and cold stimulations were applied with von- Frey filaments and acetone, respectively. To induce hyperalgesia test, radiant heat was used as thermal stimulation. The medial plantar surface of the left hind paw was used for application of the stimulations.

Experimental design

The animals were introduced to the behavioral tests at 4, 7, 14, 21 and 28 days post-surgery. The subjects got used to the test environment at least 15 minutes before beginning the test (Hamidi, Manaheji, Janahmadi, Noorbakhsh, & Salami, 2006).

Assessment of heat hyperalgesia

Plantar test device was used to evaluate paw withdrawal delay in response to radiant heat (Ugo Basile, Varese, Italy). An infrared light, as a source of heat, was transmitted from under the mid-plantar surface of the left hind paw. The delay (seconds) between the onset of the thermal stimulus and paw withdrawal was considered as thermal withdrawal latency (Banafshe et al., 2012; Bennett & Xie, 1988). A 22 seconds cut-off time was used to avoid any damage to the tissue. Each trial was performed alternately three times with an interval of 5–10 minutes to avoid sensitization for the injured and non-injured paw in the control group (Banafshe et al., 2012).

Assessment of mechanical allodynia

Mechanical allodynia was evaluated as described previously (Chen et al., 2018; Mohammadifar et al., 2021). To assess the involvement of low-threshold fibers in nociceptive behavior before and after CCI, the effects of weak stimulation of von Frey fibers (force ranging from less than 2 to 60 g) were studied. These stimulations are considered harmless because they usually provokes activity in low threshold mechanoreceptors. The rats were placed on a mesh floor (0.8 × 0.8 cm cell), shielded by a transparent plastic box (18 × 18 × 25 cm) and allowed to explore for 15 minutes or when exploratory behavior finished. Sequences of von Frey von Frey fiber stimulation were applied in a rising order of forces to the hind paw plantar surface. The von Frey filament stimulation was applied in a series of three

consecutive trials, pressing down on the hind paw until the animal withdrew its paw or the filament bent. Paw lifting was ignored due to natural locomotor behavior. The withdrawal threshold was calculated for the smallest fiber size that induced at least two withdrawal responses through three consecutive trials using the same filament. Each stimulation was applied for approximately 1 s with an interstimulus interval of 5 s.

Assessment of cold allodynia (**Acetone test**)

The modified acetone spray test was used for Cold allodynia assessment (Yoon, Wook, Sik, Ho, & Mo, 1994). While the rats stood on the perforated floor, 250 µl of acetone was sprayed onto the plantar skin using a smooth needle attached to a syringe. The trial was repeated 5 times (with an interval of 3 minutes) to the left paw. Withdrawal frequency determined as a percentage (number of withdrawals/number of trials×100) (Banafshe et al., 2014; Seltzer, Dubner, & Shir, 1990).

Statistical Methods

Two-way analysis of variance (ANOVA) was performed to the data. Tukey's test was applied as post hoc. Between groups differences were considered significant with a P value of less than 0.05. The data are presented as mean ± S.E.M.

Results

Heat hyperalgesia in the CCI group

With application of the radiant heat on the hind paw the animals lifted their feet and showed aversive behaviors such as shaking, trapping or licking the affected paw. Fig. 1A reports the heat hyperalgesia after CCI injury in the control groups. Statistical analysis indicated a significantly difference between the testing groups ($F_{3, 28}$= 99, p=0.0001). The Sham group displayed an analogous withdrawal latency with the CON animals. Also, no significant difference was evident between behavior of the CCI and CCI+Saline groups. The CCI group

significantly decreased the withdrawal latency in response to heat stimulation at days 4, 7, 21, 28 ($p<0.001$) and 14 ($p<0.01$), when compared to the sham group.

The effect of caffeine on the heat hyperalgesia

A considerable difference was found between the caffeine and vehicle treated groups ($F_{3, 28}=52.818$, $p=0.0001$). The Caf.10 group resembled the CCI+Saline group in the heat hyperalgesia test. The withdrawal latency increased at the day 4 in the Caf.50 rats ($p<0.001$). Maximum effect of caffeine was observed in the dose of 100 mg/kg where the Caf.100 group showed a considerable increased withdrawal latency at the days 7 ($p<0.001$), 14, 28 ($p<0.05$) and 21($p<0.01$) in comparison to the CCI+Saline group. We selected the dose of 50 mg/kg as the minimum effective concentration of caffeine to investigate the possible role of NO-cGMP signaling pathway. Fig. 1B illustrates the acute administration of caffeine on the pain threshold in the heat hyperalgesia.

The effect of NO-cGMP signaling pathway on the heat hyperalgesia

General statistics indicated a possible involvement of the NO-cGMP signaling pathway in the heat hyperalgesia test ($F_{3, 28}=35.78$, $p=0.0001$). Treatment with L-NAME significantly attenuated hyperalgesia at the days14 ($p<0.05$), 21($p<0.001$) and 28 ($p<0.01$) in the L-NAME group when compared to CCI+Saline group. Further, L-NAME+Caf significantly reduced hyperalgesia at the days 4 ($p<0.01$), 7 ($p<0.01$), 14 ($p<0.01$), 21 and 28 ($p< 0.001$). Moreover, compared with Caf.50 group, the L-NAME+Caf group reduced the heat hyperalgesia at the days 14 ($p<0.01$), 21 and 28 ($p<0.001$). A significant variation was also found between the L-NAME+Caf and the L-NAME groups at the day4 ($p<0.05$). Fig. 1C shows the effect of L-NAME or L-NAME+Caf on heat hyperalgesia. The data analysis showed that L-arginine or L-arginine+caffeine significantly infleunce the heat hyperalgesia threshold ($F_{3, 28}=26.312$, $p<0.001$, Fig. 1D). The animals treated with L-ARG or L-ARG+Caf showed no significant

difference in paw withdrawal latency compared to the CCI+Saline group. The L-ARG+Caf group showed a significant reduced withdrawal latency at the day 4 ($p<0.01$) when compared to Caf.50 group. L-ARG+Caf revealed not significant difference with L-ARG group.

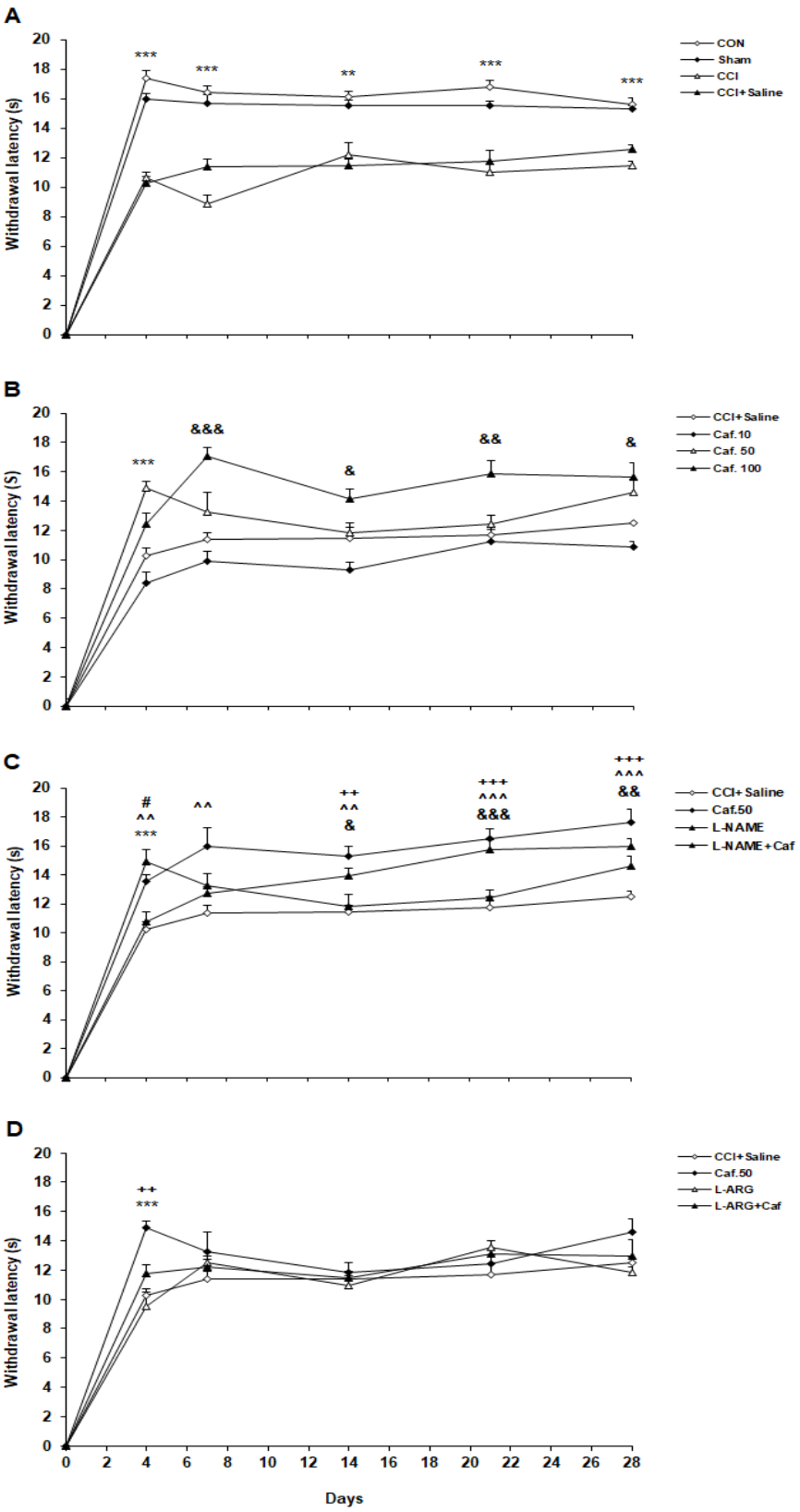

Figure 1. Heat hyperalgesia after CCI injury.

A: hyperalgesia in CCI rats and sham animals. $^{**}p<0.01$, $^{***}p<0.001$ CCI vs. sham group.

B: Effect of different doses of Caf (10, 50 and 100) on hyperalgesia, $^{***}p<0.001$ Caf.50 vs. CCI+Saline group. $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$ Caf.100 vs. CCI+Saline group.

C: Effect of L-NAME or L-NAME+Caf on hyperalgesia, $^{***}p<0.001$, Caf.50 vs. CCI+Saline group, $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}$ $p<0.001$, L-NAME vs. CCI+Saline group, $^{\wedge\wedge}p<0.01$**,** $^{\wedge\wedge\wedge}p<0.001$, L-NAME+Caf vs. CCI+Saline group, $^{++}p<0.01$, $^{+++}p<0.001$, L-NAME+Caf vs. Caf.50 group. $^{\#}p<0.05$ L-NAME+Caf vs. L-NAME group.

D: Effect of L-ARG or L-ARG+Caf on hyperalgesia, $^{***}p<0.001$, Caf.50 vs. CCI+Saline group, $^{++}p<0.01$, L-ARG+Caf vs. Caf.50 group.

Mechanical allodynia

While before surgery the subjects rarely responded even to the intense von Frey filament, however, after the CCI procedure, the ipsilateral hind paw showed to be sensitive to mechanical stimuli even with the weak sttimulations.

The statistical analysis appeared a substantial variation between the testing groups ($F_{3, 28}$= 11.96, p=0.0001). The CON and sham groups showed an analogous pattern of behavior in the mechanical allodynia test. The response to the mechanical stimulus was markedly increased in the CCI compared to sham rats at the days 21($p<0.01$) and 28 ($p<0.01$). The CCI and CCI+Saline groups also showed a similar response to this test. Fig.2A compares the results of mechanical allodynia test in different groups.

The effect of caffeine on the mechanical allodynia

A general significant difference was evident between the response of the caffeine administered groups to the mechanical allodynia test ($F_{3, 28}$=11, p=0.0001). The Caf.10 group showed a negligible difference with the CCI+Saline group in the withdrawal threshold.

However, the withdrawal threshold was increased in the Caf.50 and Caf.100 groups at the days14 ($p<0.001$) and 28 ($p<0.05$), when compared with the CCI+Saline group (Fig. 2B).

The effect of NO-cGMP signaling pathway on the mechanical allodynia

Analysis of variance indicated a significant behavioral variation when applying L-NAME ($F_{3, 28}=10.09$, $p=0.0001$). The L-NAME and L-NAME+Caf groups significantly attenuated the response to allodynia test at the days 14 ($p<0.001$) and 28 ($p<0.05$) in comparison to CCI+Saline group. However, L-NAME+Caf group showed no difference on the paw withdrawal threshold with both L-NAME and Caf.50 rats (Fig. 2C).

Statistics showed that L-arginine not considerably influences the mechanical allodynia ($F_{3, 28}=4.08$, $p=0.03$).

The animals treated with L-ARG or L-ARG+Caf did not show significant difference in paw withdrawal threshold compared to the CCI+Saline group. Moreover, there was no significant difference between L-ARG+Caf compared to L-ARG and Caf.50 groups (Fig. 2D)

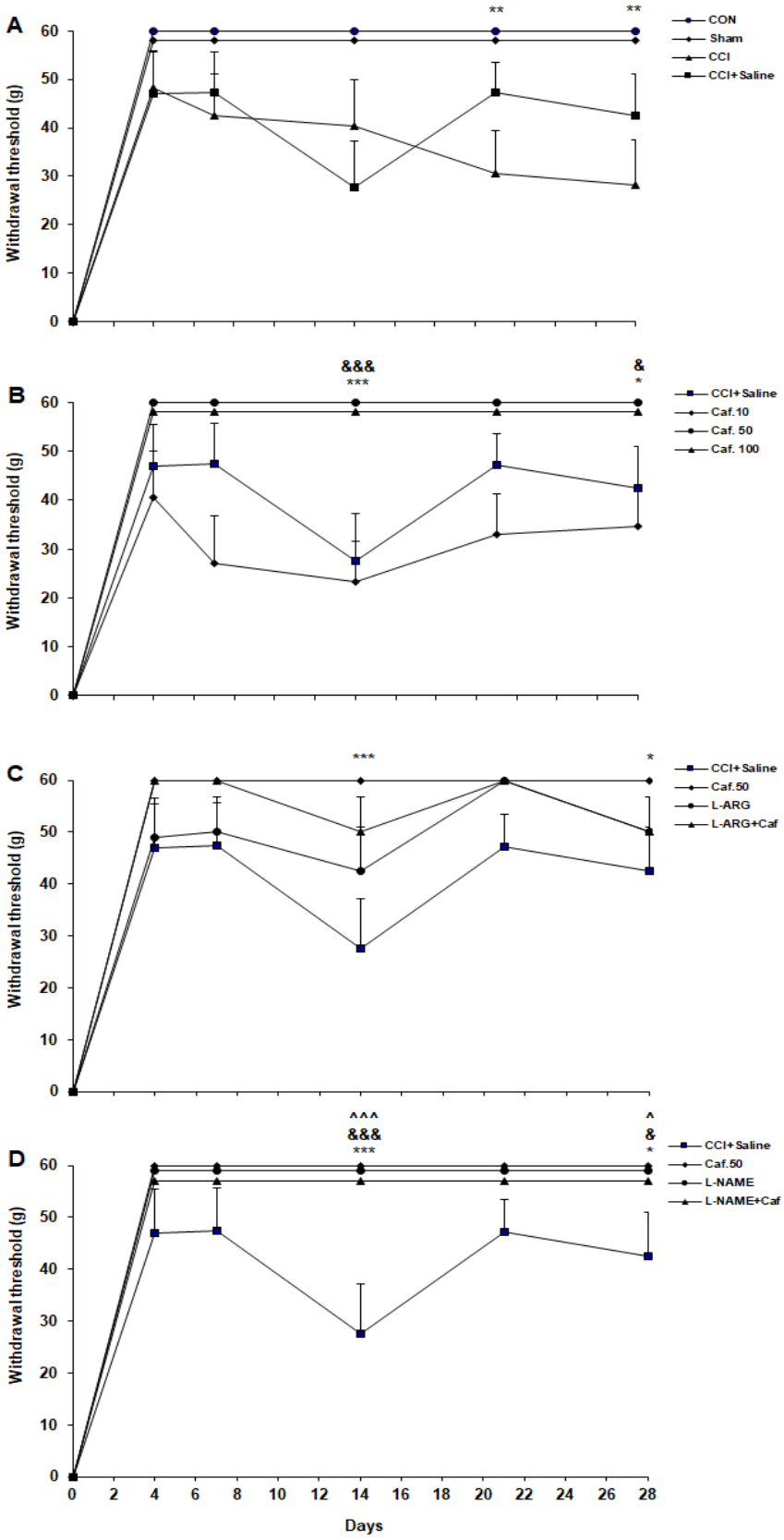

Figure 2. Mechanical allodynia after CCI injury.

A: Mechanical allodynia in CCI rats and sham animals. $^{**}p<0.01$ CCI vs. sham group.

B: Effect of different doses of Caf (10, 50 and 100) on mechanical allodynia, $^{*}p<0.05$, $^{***}p<0.001$ Caf.50 vs. CCI+Saline group, $^{\&}p<0.05$, $^{\&\&\&}p<0.001$ Caf.100 vs. CCI+Saline group.

C: Effect of L-NAME or L-NAME+Caf on mechanical allodynia, $^{*}p<0.05$, $^{***}p<0.001$ Caf.50 vs. CCI+Saline group, $^{\&}p<0.05$, $^{\&\&\&}p<0.001$ L-NAME vs. CCI+Saline group, $^{\wedge}p<0.05$, $^{\wedge\wedge\wedge}p<0.001$ L-NAME+Caf vs. CCI+Saline group.

D: Effect of L-ARG or L-ARG+Caf on mechanical allodynia, $^{*}p<0.05$, $^{***}p<0.001$ Caf.50 vs. CCI+Saline group

Cold allodynia

While the animals were not so responsive to the acetone application before surgery, however, after the CCI procedure, the ipsilateral hind paw showed a high sensitivity to acetone test. The acetone application caused rats to rapidly withdraw the affected foot (with delay of about 0.2–0.3 s) and then shook, trapped or licked it.

Statistical analysis showed a significant difference between the groups testing for the cold allodynia ($F_{3, 28}$= 151.6, p=0.0001). The results indicate that the difference of allodynia between the CON and sham groups is no significant. However, an increased withdrawal frequency was observed in the CCI compared to sham groups in testing days 4, 7, 14, 21 ($p<0.001$). The CCI and CCI+Saline groups similarly behaved in the cold allodynia test. Fig. 3A illustrates the cold allodynia in the control groups.

The effect of caffeine on the cold allodynia

The data analysis showed a substantial variation between different groups under treatment of caffeine ($F_{3, 28}$= 35.78, p=0.0001). The Caf.10 rats increased the withdrawal

frequency at the days14 ($p<0.05$), 21 ($p<0.001$) and 28 ($p<0.01$), when compared with the CCI+Saline group. However, the other caffeine treated Caf.50 and Caf.100 groups had similar response to the cold allodynia as did the CCI+Saline group (Fig. 3B).

The effect of NO-cGMP signaling pathway on the cold allodynia

The statistical evaluation showed that illustrates the effect of L-ARG or L-ARG+Caf on cold allodynia. Analysis of variance showed that L-arginine administration significantly underlies the withdrawal frequency in the cold allodynia testing ($F_{3, 28}=10.08$, $p=0.0001$). The L-ARG group showed no significant difference in withdrawal frequency compared to the CCI+Saline group. The L-ARG+Caf animals noticeably reduced the withdrawal frequency at the days 4 ($p<0.01$), 7 ($p<0.05$) and 21 ($p<0.05$) compared to CCI+Saline group. The L-ARG+Caf rats appeared a significant reduction in the withdrawal frequency at the days 4 ($p<0.01$), 7 ($p<0.001$) and 21 ($p<0.01$) compared to the Caf.50 group. Also the L-ARG+Caf animals displayed a marked reduced the withdrawal frequency at the days 4 ($p<0.05$) compared to L-ARG group (Fig. 3C).

We found that the L-NAME application was effective on the mechanical allodynia ($F_{3, 28}=20.02$, $p=0.0001$). Treatment with L-NAME significantly attenuated the withdrawal frequency at the days 4 ($p<0.05$) and 7 ($p<0.01$) in the L-NAME compared to CCI+Saline group. Also, the L-NAME+Caf group significantly reduced the withdrawal frequency at the days 4 ($p<0.05$), 7 ($p<0.01$), 14 ($p<0.01$) and 21 ($p<0.05$) compared to CCI+Saline group. Moreover, L-NAME+Caf significantly reduced allodynia at the days 4 and 21($p<0.01$), 7, 14 and 28 ($p<0.001$) when compared with Caf.50 group (Fig. 3D).

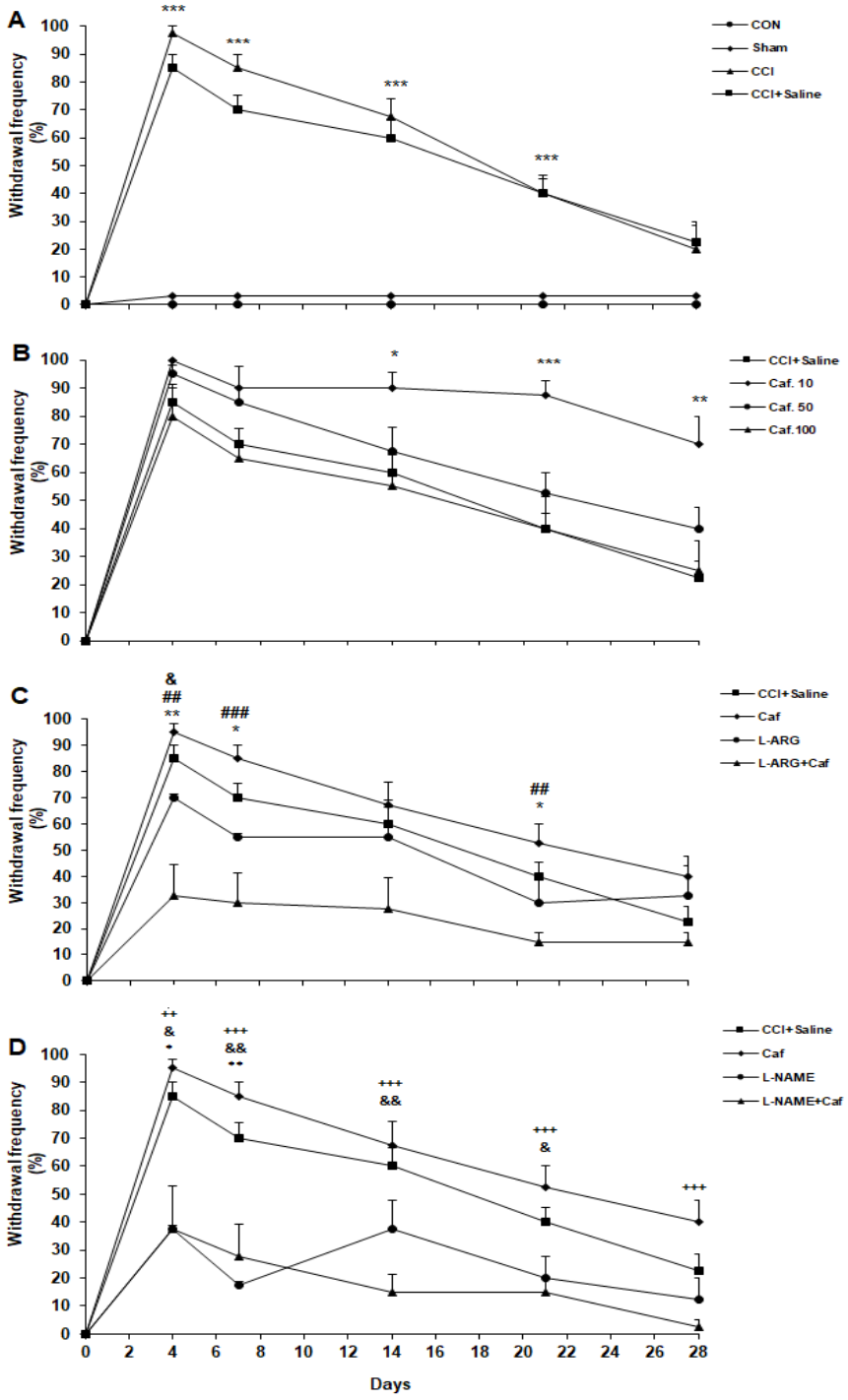

Figure 3. Cold allodynia after CCI injury.

A: Cold allodynia in CCI and sham animals. $^{***}p<0.001$ CCI vs. sham group.

B: Effect of Caf (10, 50, and 100) on cold allodynia, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ Caf.10 vs. CCI+Saline group.

C: Effect of L-ARG or L-ARG+Caf on cold allodynia, $^{*}p<0.05$, $^{**}p<0.01$, L-ARG+Caf vs. CCI+Saline group, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, L-ARG+Caf vs. Caf.50 group, $^{\&}p<0.05$ L-ARG+Caf vs. L-ARG group.

D: Effect of L-NAME or L-NAME+Caf on cold allodynia, $^{*}p<0.05$, $^{**}p<0.01$, L-NAME vs. CCI+Saline group, $^{\&}p<0.05$, $^{\&\&}p<0.01$ L-NAME+Caf vs. CCI+Saline group, $^{++}p<0.01$, $^{+++}p<0.001$, L-NAME+Caf vs. Caf.50 group.

Discussion

The finding of this study indicated that caffeine at a dose of 10 display a pronociceptive effect, conversely, the doses of 50 and 100 of caffeine show antinociceptive effect. Whereas L-NAME alone or pre-treated with caffeine has antinociceptive effect, L-ARG pre-treatment with caffeine increased heat hyperalgesia and decreased cold allodynia.

Caffeine is an antagonist of ARs and its main targets are A_1 and A_{2A} subtype receptors (Carrillo & Benitez, 2000). Adenosine displays its analgesic role by activation of A_1 receptors in the nervous system. On the other hand, A_{2A} receptor shows both pronociceptive and antinociceptive effects (J Sawynok, 2016; Vincenzi, Pasquini, Borea, & Varani, 2020; Yamamoto et al., 2003; Zahn, Straub, Wenk, & Pogatzki-Zahn, 2007). A1 receptors are found on peripheral sensory nerve endings in the dorsal horn of spinal cord as well as supraspinal structures involved in pain-processing (Vincenzi et al., 2020). A_2 receptors are located on inflammatory and immune cells and are considered as targets for inflammatory and immune conditions (J Sawynok, 2016). In the CNS, they are expressed in pre and post-synaptic neurons

in brain (Antonioli et al., 2014; Popoli & Pepponi, 2012). The results of this study show that the lowest dose of caffeine had a pronociceptive effect and increased the cold allodynia. Consistent with our findings, it is shown that 1 to10 mg/kg of caffeine increases hyperalgesia and allodynia (Esser & Sawynok, 2000; Wu et al., 2006). Moreover, heat hyperalgesia was exacerbated in the A_1 receptors knockout mice (Wu et al., 2005). The hyperalgesic effects of the minimum dose of caffeine may be due to antagonizing effect on A_1 receptors (Jana Sawynok, Reid, & Fredholm, 2008, 2010). We showed that acute administration of caffeine at the doses of 50 and 100 mg/kg reduces the heat hyperalgesia and mechanical allodynia. Numerous studies confirm our results in that some doses of caffeine show antinociceptive effect (López et al., 2006; Shapiro, 2008; Wu et al., 2006). Blockade of A_{2A} receptors is one of the proposed mechanisms of analgesic effects of caffeine. Consistently, hypoalgesia was reported in mice lacking the A_{2A} receptors (Ledent et al., 1997). Both central and peripheral A_{2A} receptors are involved in pain facilitation and play an important pronociceptive role (J Sawynok, 2016). It has been proposed that caffeine at a dose of 100 mg/kg has more inhibitory effect on A_{2A} receptors. In this context, caffeine revealed a dose-dependent anti-hyperalgesia and the maximal effect was achieved at a dose of 100 mg/kg. Overall, our findings suggest that effect of caffeine on hyperalgesia to be partly due to its dose-dependent effect on A_1 receptors or A_{2A} receptors (Jana Sawynok et al., 2010; Wu et al., 2006). Caffeine can modulate pain mainly through antagonism of adenosine receptors (Davis & Green, 2009; Massey, Bergman, Wise, & Sherrard, 1994; Ribeiro & Sebastiao, 2010). Activation of A_{2A} receptors leads to increased production of NO, therefore, antagonizing effect of high dose caffeine (50 and 100 mg/kg) may leads to decreased production of NO (Esmaili & Heydari, 2019). Some evidence indicate that NO is involved in the development of N (Chauhan, Sheng, Hu, Prasad, & Lokensgard, 2018; Miller, Miller, & Malfait, 2014; Zhao et al., 2018). To further investigate the role of the NO-cGMP pathway, we applied L-arginine as NO releasers and L-NAME (non-

selective NOS inhibitor). Administration of L-NAME before caffeine decreased susceptibilities to heat hyperalgesia, cold and mechanical allodynia. Also, L-arginine administration before a caffeine increased heat hyperalgesia. These findings suggest that the pain attenuation effects of L-NAME might be mediated through inhibitory effect on NOS isoforms and subsequently, NO production. Another proposed mechanism to explain antihyperalgesia effect of caffeine is reduction of cyclooxygenase (COX-2) and prostaglandin E (PGE2). In this context, caffeine inhibits the synthesis of PGE2 and COX-2 in rat microglial cells (Fiebich et al., 2000). In addition, caffeine potentiates the antihyperalgesic effects of nonsteroidal anti-inflammatory drugs (NSAIDs)(Abou-Atme, Melis, & Zawawi, 2019). Hyperalgesia is mostly associated with increased expression of COX-2 and a local production of PGE2 (Li et al., 2018). Inhibitory effect of caffeine on COX-2 and reduced synthesis of PGE2 may contribute to the antihyperalgesic effect of caffeine (Fiebich et al., 2000). Interestingly, there is an interaction between NO and PGE2, so that NO increases the production of PGE2 (Ilari et al., 2020).

In conclusion, our findings suggest that decreased NO production in the presence of caffeine may contributes to reduction of PGE2 and subsequent antihyperalgesia. Involvement of the NO-cGMP pathway in the pain threshold modification might be a possible mechanism of caffeine. Elucidating the cause of the opposite effects of different doses of caffeine on pain requires further research.

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Conflict of interest

No conflict of interest.

Author contribution

A Heydari designed the study. A Haydari and GA Hamidi led the work. M Naderi and S Nasrollahi performed the experiments and collected the data. F Aghighi assisted in writing the manuscript. A Heydari and GA Hamidi wrote the manuscript. M Salami reviewed and edited the final version of the manuscript.

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