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Effects of Single and Long-Term Treatment with Caffeine on the Chronic Unpredictable Stress-Induced Changes in Nociception in Rodents

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ABSTRACT

Background and Objective: There is sufficient data that Chronic Unpredictable Stress (CUS) induced changes in nociception using different animal models. CUS is considered as a valuable animal model to study depression. Adenosinergic system plays a key role in pain transmission in different brain areas interacting with its receptors and other neurotransmitters. The effects of caffeine as a non selective adenosine receptor antagonist on the chronic unpredictable stress-induced changes in nociception using two experimental models of phasic and tonic pain was studied. **Methods:** CUS induced depressive-like condition; paw pressure test (phasic pain model) and writhing test (tonic pain model) were used. Methylxanthine caffeine was administered acutely (2, 20 and 40 mg kg^{-1}) and long-term (8 mg kg^{-1} day kg^{-1}, 4 weeks). Desipramine (10 mg kg^{-1} day^{-1}, 4 weeks) was used as a reference antidepressant. **Results:** Single caffeine injection showed bidirectional effects, enhancing of CUS-induced antinociception in phasic model and abolishment of CUS-induced pro nociception in tonic model. Long-term caffeine administration *per se* increased nociception and similarly to the antidepressant desipramine was able to abolish the CUS-induced antinociception in paw pressure test in rats. **Conclusion:** The results underlined the different impact of mood depression on phasic and tonic acute pain and revealed the ability of caffeine to reverse CUS-induced changes in nociception depending on the duration of administration.

Key words: Depression, phasic and tonic acute pain, adenosine, rats, mice

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INTRODUCTION

Exposure of animals or humans to different stressors caused a sequence of biochemical, physiological and behavioral changes that might be a pre requisite to development of depressive disorder. Many of the acute stressful stimuli have been shown to induce an analgesia activating multiple pain-inhibitory systems as well as potentiated the effects of some well-defined analgesics[1,2,3]. Unlike the acute stress-induced changes, chronic stress may provoke opposite effects on the nociception[4,5]. Some clinical studies showed that depression and pain are two interrelated health problems that can occur together and originate from one another since depression may be a reason for chronic pain and vice versa. Chronic pain conditions are often predisposition for occurrence of depression[6,7]. This is a presumption of a common theory which assumes that depression and pain follow the same descending pathways of the central nervous system. In this regard, preclinical experiments using Chronic Unpredictable Stress (CUS) are liable animal model of depression[8] which showed that depression aroused visceral hyperalgesia that is activated by innate immune system[9].

Adenosine and its receptors can be found in diverse brain are as being one of the most widely distributed neuromodulator in central nervous system[10] which play an important role in the regulation of mood and nociception. The adenosine and its analogs have a

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complex role in the transmitting of nociceptive information at the different levels in the periphery and the central nervous system. Systemic, intrathecal or intra-cerebroventricular administration of A_1 receptor agonists produced analgesic effects in variety of experimental pain models while A_2 receptors in the periphery are responsible for a pronociceptive effect of adenosine[11,12]. Methylxanthine caffeine which is a non-selective adenosine A_1/A_2 receptor antagonist, is the most widely used psycho stimulant, food ingredient and adjuvant to different analgetics[13]. There is evidence that caffeine produced an antinociception in animal models[14,15] and in human[16]. Moreover, caffeine similarly to established antidepressants may ameliorate desperate-like behavior in the forced swimming test and decreased the immobility time during the test procedure[17].

The present study was aimed to elucidate the role of endogenous adenosine in the CUS-induced changes in nociception through comparing the effects of single and chronic treatment with adenosine receptor antagonist caffeine.

MATERIALS AND METHODS

Animals: The experiments were carried out on male young-adult Wistar rats weight of 200-300 g (used in paw-pressure test) and male young-adult ICR mice weight 25-30 g (used in writhing test), obtained from the animal breeding facility of the Bulgarian Academy of Sciences. All animals were housed individually under equal standard conditions: 21±1°C, 40-50% humidity, 12 h light-dark cycle (light on 08:00 h) with free access to food and water and daily handled for a period of 10 days before tests (habituation period). All experiments were carried out between 10:00 am and 13:00 pm during the autumn and were approved by local ethic committee of Institute of Neurobiology, Bulgarian Academy of Sciences which are fully in accordance with EC Directive 2010/63/EU for animal experiments.

Experimental design: Animals were divided in groups: A) control and B) Chronic Unpredictable Stress (CUS) treated with subgroups depending of the dose used, each with n = 10: (1) Treated with saline (2) Acute caffeine at doses 2, 20 and 40 mg kg^{-1} (3) Long term caffeine at a dose of 8 mg kg^{-1} day^{-1} for 4 weeks and (4) Long term desipramine at a dose of 10 mg kg^{-1} for 4 weeks. Methylxanthine caffeine (Sigma-Aldrich) was dissolved in saline and administered by an oral gavage in a volume of 0.1 mL/100 g of body weight. Desipramine hydrochloride (DMI; Sigma-Aldrich) was administered intraperitoneally in the same volume. Saline groups were treated either orally (n = 5) or intraperitoneally (n = 5). Data from the control groups did not show any differences and they were combined. The drugs (caffeine or DMI) were administered at 10:00 h, 30 min before the beginning of CUS procedure, because the half-lives of caffeine for doses lower than 10 mg kg^{-1} range from 0.7-1.2 h in rats[18]. The CUS procedure was started after the period of adaptation and was continued for 6 -2 weeks before and 4 weeks along with the drug (or saline) treatment. During this period, non-stressed animals were left undisturbed in their cages. Behavioral experiments were conducted during the 6th week after the beginning of the CUS, from 08:30 to 10:00 h, before daily drug and/or CUS treatment.

Chronic Unpredictable Stress (CUS): The CUS was based on the procedure originally designed by Willner *et al*.[8]. In brief: animals subjected to CUS were exposed to variable stressors: light switched on during the dark time, foot shock (10 shock procedures of 2 mA on a grid floor with a shock interval of 2 sec), food or water deprivation overnight (16 h), exposure of a resident animal to a unknown cage mate, wet bedding (400 mL cool tap water) for 24 h, 60 min restraint stress, home cages tilted vertically at 30°C for 24 h. The stressors were applied daily in a random order to avoid habituation for a period of 6 weeks. Recently, confirmed depressive effects of CUS procedure have been confirmed[19].

Acetic acid-induced writhing test in mice: Writhing test was selected as a model of acute tonic pain[20]. Acetic acid at a concentration of 1% was administered intraperitoneally (IP) in an injection volume of 0.1 mL/10 g of body weight. Immediately after injection of the irritant, the mice were placed in individual transparent cages and the number of specific abdominal constrictions (writhes) of each mouse was summarized at 5 min intervals for 30 min. The mice with decreased number of writhes were considered protected by the test agent.

Paw-pressure test for nociception: The paw pressure withdrawal reflex was selected as a model of phasic pain and was determined by an analgesiometer (UgoBasile, Italy). The mechanical pressure (in grams) required eliciting pain responses such as withdrawal or struggle was established as mechanical pain threshold. The cut-off pressure 450 g was maintained to avoid paw injury. The testing was optimized by single training of the animals 1 day before the experiments without applying a pressure[21].

Statistical analysis and graphics preparation: Data were analyzed by two-way ANOVA (factors CUS and Drug-Caffeine or Desipramine) followed by *post hoc* Bonferroni test. For samples that failed normality test, a non-parametric Mann-Whitney test was employed. The $p<0.05$ was accepted as an index of statistically significant differences. The graphics were prepared using Program SigmaPlot 11.0.

RESULTS

Single oral administration of caffeine at doses of 2, 20 and 40 mg kg^{-1} in control groups rats did not change the paw pressure threshold vs. vehicle treated groups (Fig. 1). After six weeks of exposure to CUS, however, it was that saline-treated animals developed an anti-nociception in this somatic pain model (factor CUS-F 1, 67 = 43.521, $p<0.001$, Fig. 1). Single caffeine administration before the exposure to the last stressor at doses of 2 and 40 mg kg^{-1} potentiated the CUS-induced antinociception (factor Caffeine-F 1, 67 = 4.112, $p<0.01$ and interaction between factors CUS×Caffeine-F 1, 67 = 3.411, $p<0.02$, Fig. 1).

In writhing test, only highest dose of 40 mg kg^{-1} was able to increase the number of writhes in the control non-stressed group, producing a pronociceptive effect (q = 6,929, $p<0.001$)(Fig. 2). The same period of CUS treatment, however, leaded to an increased nociception in visceral pain model (factor CUS - F 1, 65 = 4,898, $p<0,030$, Fig. 2). Caffeine at doses of 2 and 40 mg kg^{-1} returned the number of writhes to the control level (interaction between factors CUS×Caffeine-F 1, 65 = 11.304, $p<0.001$, Fig. 2).

Long term treatment with caffeine significantly decreased the paw pressure threshold in non-stressed group (factor Caffeine-F 1, 44 = 19.032, $p<0.001$, Fig. 3). Moreover, a parallel long term treatment with caffeine together with CUS significantly diminished the CUS-induced antinociception (t = 4,350, $p<0.001$, Fig. 3). The referent antidepressant used showed a similar effect on CUS-induced increase of threshold in paw pressure test (factor Desipramine-F 1, 44 = 13,851, $p<0.001$ and interaction between factors CUS× Desipramine-F 1, 44 = 19,909, $p<0.001$, Fig. 3).

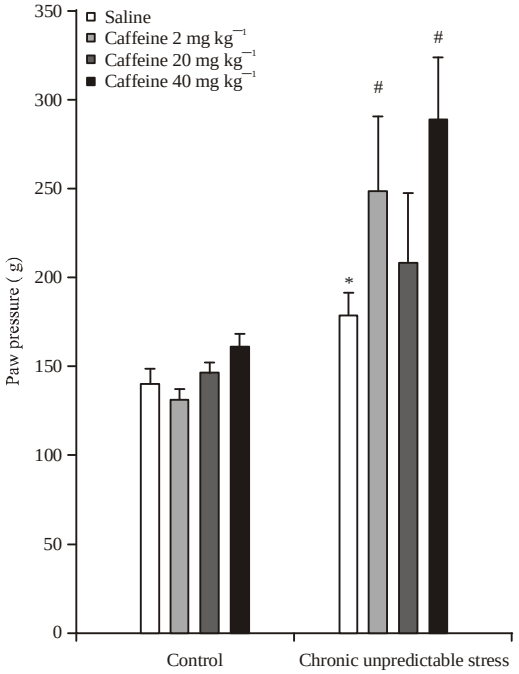

Fig. 1: Effect of caffeine given acutely at doses of 2, 20 and 40 mg kg^{-1}, p.o. on the nociception in paw pressure test in healthy (Control) and depressed after Chronic Unpredictable Stress (CUS) rats. Data showed Mean±SEM. ⋆$p<0.05$ vs the controls (saline); #$p<0.05$ vs. CUS (saline) group

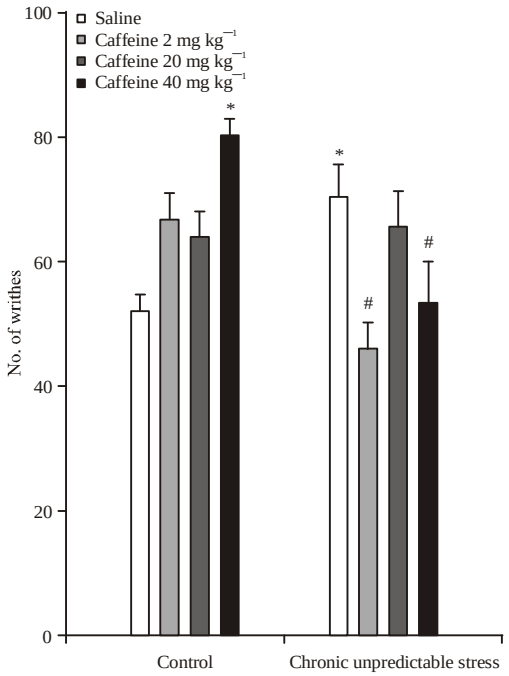

Fig. 2: Effect of caffeine given acutely at doses of 2, 20 and 40 mg kg^{-1}, p.o. on the nociception in writhing test in healthy (Control) and depressed after Chronic Unpredictable Stress (CUS) mice. Data showed Mean±SEM. ⋆$p<0.05$ vs the controls (saline); #$p<0.05$ vs CUS (saline) group

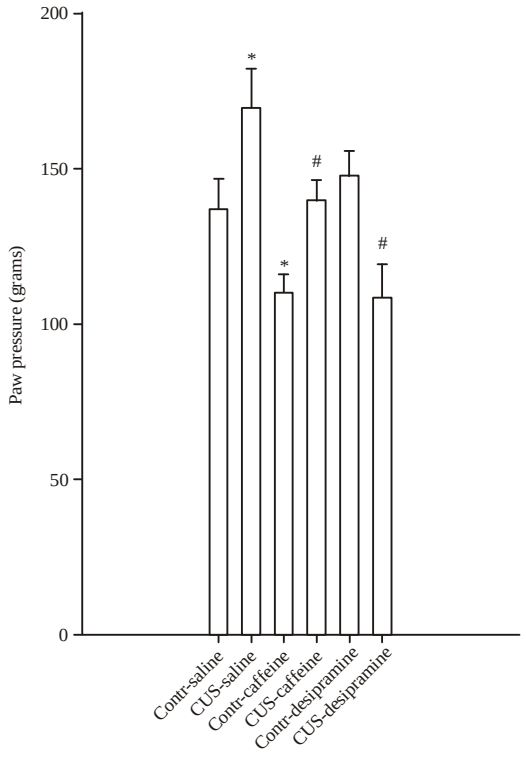

Fig. 3: Effect of long term caffeine administration at dose of 8 mg kg^{-1} day^{-1}, p.o. during 28 days on the nociception in paw pressure test in healthy (Control) and depressed after Chronic Unpredictable Stress (CUS) rats. Desipramine was used as a referent antidepressant given i.p. at a dose of 10 mg kg^{-1} day^{-1} for 28 days. Data showed Mean±SEM $^{\star}p<0.05$ vs the controls (saline); $^{\#}p<0.05$ vs CUS (saline) group

DISCUSSION

The present results showed that CUS procedure provoked bidirectional effects-an antinociception in phasic pain and a pronociception in tonic pain. The data confirmed previews publications on the stress-induced decrease in phasic pain sensitivity[22,23]. Such effect was established likewise in a chronic model of neuropathic pain[24]. There are some putative mechanisms suggested, including loss of "motivation" for withdrawal or escaping from the noxious stimuli and motor deficit. This behavioral effect might be a result of central dopaminergic system impairment, which resulted in delay response in phasic pain. On the other hand, decreased monoamine levels in depressed subjects may reflect on the decreased activity of descending pain inhibitory system and triggering of hypersensitivity to nociception. The impact of depression on the levels of different monoamines in the brain may explain, least in part, the divergent effects of CUS-antinociception in phasic pain model and pronociception in tonic pain. The assumption for a leading role of serotonin and norepinephrine in the co-morbidity of depression and pain syndrome was supported by data for some dual-action antidepressants which showed to be effective in managing the pain symptoms associated with depression[25]. Further data showed that depression is closely related to cognition and memory dysfunction which may partly diminish attention and decrease responses to noxious stimulation. Recently, it was shown that chronic stress and depression lead not only to drop in 5-HT level and 5-HT receptor sensitivity but also decreased significantly adult neurogenesis and damaged hippocampus[26]. Hippocampus as the main integral component of limbic system takes a part both in the development of depressive-like behavior and processing of pain-related information. Further, both chronic stress and chronic pain resulted in hippocampal activation and NK1 receptor down-regulation[27]. According to other studies[5], in the present study it had been shown that stress procedure is accompanied with an increase in visceral noxious sensitivity. Additionally, recently published data showed that CUS increased chronic widespread nociception induced by multiple injections of nerve growth factor[28]. These data support the hypothesis for common mechanisms and co-morbidity of pain and depression but exact mechanism which determines different impact of experimental depression on phasic and tonic acute pain remains still unclear.

The data of present study showed that single doses of caffeine did not influence phasic pain response but given in the same doses it was able to enhance in CUS-induced antinociception. It is well known that the motor-activating effects induced by administration of caffeine in rats involve central blockade of both A_1 and A_{2A} receptors[29], however recently published data showed that CUS- induced depressive-like behavior is associated with increased A_1 receptor binding and immunoreactivity in hippocampus and A_{2A} binding in the striatum which supports the idea for the participation of adenosinergic system in the different manifestations of depression[30]. The previous data showed that both A_1 and A_{2A} receptor agonists have antinociceptive effect in the same model of visceral pain[31]. The effects of caffeine on CUS-induced changes of nociception, however, were bidirectional depending to the type of acute pain. While in phasic pain, it potentiated depressive antinociception,

in tonic pain, caffeine abolished stress-induced increase of nociception. Considering the role of A_1 receptors in adenosinergic anti nociception, there was a small possibility for participation of this type of receptors in caffeine- induced potentiating of antinociception. It is more likely that A_{2A} receptor antagonism is involved in caffeine effect in phasic pain. Although, The previous data showed that the main metabolite of the caffeine, theophylline, realized an antinociceptive effect due to interaction between A_1 and alpha-2-adrenergic receptors[32]. In the present work, it had been shown that chronic caffeine administration, similarly to adrenergic antidepressant desipramine, was able to abolish CUS-induced antinociception in somatic pain. Moreover, existing data showed that chronic caffeine increased the levels of endogenous monoamines norepinephrine, dopamine and serotonin as well as the adenosinergic, alpha adrenergic and serotonergic receptors in rat brain[33,34].

Taken together, these results underlined the different impact of mood depression on phasic and tonic acute pain and revealed the ability of caffeine to reverse CUS-induced changes in nociception depending on the duration of administration.

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