

**EFFECTS OF ADENOSINE RECEPTOR LIGANDS ON THE NOCICEPTION AND STRESS-INDUCED ANTINOCICEPTION IN MICE.**

**Lubomira Ivanova\*, Daniela Pechlivanova\*\*, Elena Dzhambazova\*\*\*, Vasil Georgiev\*\***

*\* Faculty of Biology, Sofia University "St. Kl. Ohridski", 8 Dragan Tsankov Blvd., 1164, Sofia, Bulgaria*

*\*\*Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 23, 1113 Sofia, Bulgaria*

*\*\*\* Faculty of Medicine, Sofia University "St. Kl. Ohridski", 1 Kozia Str., 1407 Sofia, Bulgaria*

**Abstract**

**Introduction:** It is well known that exposure to different stressors caused a sequence of biochemical, physiological and behavioural changes including analgesia which is realised by activating multiple endogenous pain inhibitory systems. Although the existing data reveal the important role of the adenosine modulatory system in the processing of nociceptive information, scarce data are available for its role in stress-induced changes in nociception.

**Materials and Methods:** Male ICR mice – controls and exposed to acute (120 minutes) restraint stress (RS) were used; Acetic acid-induced nociception (writhing test); Adenosine A<sub>1</sub> receptor agonist N<sup>6</sup>-R phenylisopropyl-adenosine (R-PIA, 0.5 mg/kg, intraperitoneally, IP), nonselective adenosine receptor antagonist theophylline (acute 75mg/kg, IP and chronic 75 mg/kg/day/14 days, IP).

**Results:** RS and R-PIA decreased the visceral nociception in mice. Acute and chronic treatment with theophylline did not influence the pain reactions neither in nonstressed nor in stressed animals. R-PIA administered before restraint stress fully reversed stress-induced antinociception. Single dose of theophylline antagonized the effects of R-PIA both in nonstressed and stressed animals. Chronic theophylline did not antagonize the antinociceptive effect of R-PIA, but abolished R-PIA induced reversion of SIA.

**Conclusion:** Endogenous adenosine is not able to change the RS-induced visceral antinociception, as theophylline did not show any effects on the stress. However, adenosine A<sub>1</sub> receptors are involved both in the antinociception in the healthy mice and RS-induced antinociception, perhaps by different mechanism.

**Key words:** Adenosine, R-PIA, Theophylline, Restraint stress, Nociception

**Introduction**

It is well known that exposure to different stressors caused a sequence of biochemical, physiological and behavioural changes. Many of stressful stimuli induce analgesia by activating of multiple endogenous pain inhibitory systems or potentiated the effects of some well-defined analgesics [1, 2].

The purine adenosine is a representative of the neuromodulatory systems because it is not stored in vesicles nor released as a bolus in response to depolarization of a presynaptic membrane. As a by-product of ATP, ADP and cAMP metabolism it accumulates and can bind to the selective pre- and postsynaptic adenosine receptors and exerts neuroprotective, anticonvulsant and analgesic effect [3, 4, 5]. Its effects are result of the activation of adenosine A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors, which are widely distributed in the central nervous system with highest density of A<sub>1</sub> receptors on peripheral sensory nerve endings, specific spinal and supraspinal sites involved in the processing of the pain signaling [6], and structures with high levels glucocorticoid receptors like hippocampus and cerebral and cerebellar cortex [7]. Adenosine A<sub>1</sub> receptor activation decreases GABA transmission in periaqueductal grey and diminished activity of periaqueductal grey-rostral ventromedial medulla axis, a system rich in opioid receptors and critical for mediating endogenous analgesia [5]. Adenosine A<sub>2A</sub> receptors are expressed peripherally on inflammatory and immune

cells, exerting anti-inflammatory actions, and they have a more restricted distribution in CNS mostly found in striatum colocalized with dopamine D<sub>2</sub> receptors in GABA-ergic medium-sized neurons that also contain enkephalin [8], while the activation of A<sub>2B</sub> receptors produces pronociceptive or pain-enhancing effects at peripheral sensory nerve terminal level in rodents [9]. A<sub>1</sub> receptors are localized also in microglia where they contribute to the antinociception inhibiting pain-provoked glial activation and hypertrophy [10]. All types of adenosine receptors belong to the G-protein coupled superfamily as A<sub>1</sub> and A<sub>3</sub> couple predominantly to G proteins of the Gi family whereas the two A<sub>2</sub> receptors predominantly couple to members of the Gs family. It has been established that physiological response to adenosine is a result of balance between A<sub>1</sub> and A<sub>2</sub> receptor activations and the stimulatory effects of A<sub>2</sub> receptors can be masked by activation of A<sub>1</sub>, respectively. Binding of adenosine to A<sub>1</sub> receptors on presynaptic membranes inhibits the release of most brain neurotransmitters, including glutamate, GABA, norepinephrine, serotonin and acetylcholine [11]. Cellular mechanisms of action of A<sub>1</sub> receptors include inhibition of cyclic AMP/PKA and interactions with Ca<sup>2+</sup> and K<sup>+</sup> channels, interactions with the PLC/IP3/DAG pathway [5].

Methylxanthines, non-selective adenosine A<sub>1</sub>/A<sub>2</sub> receptor antagonists, inhibited adenosine-induced antinociception, however the methylxanthine caffeine, was able to produce antinociception in animal experimental models and in humans as well as to improve the effects of some analgetics [12, 13]. Moreover, the chronic administration of caffeine increased the sensitivity of mice to the antinociceptive action of A<sub>1</sub> receptor agonist R-PIA as was shown in tail flick test in rats [14].

Although existing data characterize the important role of adenosine modulatory system in the processing of nociceptive information, there are only several publications about its participation in stress-induced antinociception (SIA) [15]. The aim of the present study is to elucidate the role of adenosine A<sub>1</sub> receptor activation and A<sub>1</sub>/A<sub>2</sub> receptor blocking in the stress-induced changes in visceral nociception.

### Material and methods

*Animals and housing.*: The experiments were carried out on male albino mice ICR strain (18–20 g) bred in an air-conditioned room at a temperature of 24 ± 1°C with food and water available *ad libitum* except during the experiments. All tests were conducted between 09:00 and 12:00 h and were carried out in accordance with the institutional guidance and general recommendations on the use of animals for scientific purposes.

*Drugs and treatment.* Selective adenosine A<sub>1</sub> receptor agonist N<sup>6</sup>-R phenylisopropyladenosine (R-PIA, 0.5 mg/kg) and nonselective adenosine receptor antagonist theophylline (1,3-dimethylxanthine, acute 75mg/kg, and chronic 75 mg/kg/day/14 days), both obtained by Research Biochemicals International, were dissolved in saline and injected intraperitoneally (i.p.) in a volume of 1 ml/kg b.w. The equivalent volume of vehicle and acetic acid (diluted with distilled water to a concentration of 1%) were administered i.p. to the control and experimental groups respectively. Theophylline (acute) was injected 20 minutes before R-PIA and R-PIA was injected 15 min the before acetic acid. The tests in chronic treated theophylline group were carried out 48 hours after the last injection (wash out period).

*Restraint stress (RS).* It was induced by placing the mice in plastic tubes (25 mm inner diameter and 10 cm long) with suitable ventilation at one end and with the other side closed off by plastic tape so that the animals were unable to move for 2 hours.

*Acetic acid-induced nociception (writhing test).* Immediately after injection of the acetic acid, the mice were placed in individual cages and the number of specific abdominal constrictions (writhes) of each mouse was summarized at 5-min intervals for 30 minutes. The mice with decreased number of writhes were considered protected by the test agent [16].

*Statistical analysis.* The data were analyzed by a multifactor analysis of variance (ANOVA), followed by the Bonfferoni post test.  $P<0.05$  were considered as significant.

### Results

Adenosine A<sub>1</sub> receptor agonist R-PIA significantly diminished the number of writhes during the whole 30<sup>th</sup> min period of observation ( $t = 4.819$ ,  $P<0.001$ ) (fig. 1). Theophylline administered acutely at a dose of 75 mg/kg showed an antinociceptive effect only during first 5 minutes of observation (data no shown) however, pre-treatment with the methylxanthine abolished the antinociceptive effect of R-PIA ( $p<0.05$ ) (fig. 2 and 3).

Exposure to RS provoked a significant decrease of visceral pain reactions ( $t = 5.388$ ,  $P = <0.001$ ). Injected 15 minutes prior acute restraint stress the adenosine analogue R-PIA fully reversed stress induced analgesia ( $t=5.918$ ,  $P<0.001$ ) (fig. 1). Pre-treatment with a single dose theophylline before the stress exposure did not change SIA however it abolished the effect of R-PIA (fig.2).

Chronic treatment with the same dose of theophylline did not influence the pain reactions neither in nonstressed nor in stressed animals (fig.2). Chronic theophylline pretreatment did not change the antinociceptive effect of R-PIA in non-stressed mice, but abolished the effect of R-PIA on SIA (fig.3).

### Discussion

The results showed that the selective adenosine A<sub>1</sub> receptor activation produced an antinociception in used visceral model of nociception that was successfully antagonized by single dose of theophylline. This data are in accordance with our previous study where we have shown that both A<sub>1</sub> and A<sub>2</sub> receptors take a part in the inhibition of visceral pain using the same experimental model [12]. It is well known that adenosine and its analogs produce analgesic effects after systemic, intrathecal or intracerebroventricular administration in different pain tests in rats and mice [17]. Adenosine exerted its intrinsic antinociceptive activity through various intracellular mechanisms: i/ peripherally by inhibition of c AMP formation and PKA, as well as NO/cyclic GMP/PKG/KATP signaling; ii/ in the spinal cord by presynaptic inhibition of excitatory amino acid and neuropeptides release, post-synaptic hyperpolarization and reduced sensitization; iii/ in the supraspinal brain structures adenosine A<sub>1</sub> receptors produced a hyperpolarization through activation of K<sup>+</sup> channel, inhibited GABA transmission in PAG, suppressed the activity of ON-cells and activate OFF-cells in PAG-RVM axis [5]. Moreover, the spinal release of adenosine is a component of opioid drug efficacy. Additionally, there are data suggesting that adenosine is involved in mediation of morphine and noradrenaline-induced antinociception [18]. Noradrenergic neurotransmission, on the other hand, is included in the adenosinergic regulation of nociception since alpha-2- adrenoceptor antagonist diminished the theophylline- induced antinociception in a phasic pain model in rats [19].

Enhanced opioid-induced analgesia in rats exposed to restraint stress has been well documented [1]. Experimental evidence showed that acute stress induces a release of endogenous opioids that contribute to this augmentation [20]. Similarly to opioids, RS exposure increased the adenosine A<sub>2</sub> receptor agonist 5'-N-ethylcarboxamido adenosine (NECA)-induced anti-

nociception. Supporting the assumption of a common mechanism of SIA, involving opioid and adenosine receptors, there are data that both the nonselective adenosine receptor antagonist caffeine and opioid antagonist naltrexone could abolish the stress-induced potentiation of adenosine antinociception [21]. Our present results have confirmed that the RS is able to induce visceral antinociception. This effect was neither blocked nor potentiated by the antagonist adenosine theophylline. The activation of A<sub>1</sub> adenosine receptors by the selective agonist R-PIA, however, abolished RS-induced antinociception. Acute theophylline did not influence the stress-induced changes in pain reactions, but fully reversed the effect of R-PIA on the RS-induced antinociception. These data corroborated the role of A<sub>1</sub> receptors in the regulation of stress response only if they are activated additionally with the selective analogue. The methylxanthine blocked proportionally both A<sub>1</sub> and A<sub>2</sub> adenosine receptors and their effects on the different functions dependents mainly on the receptor distribution. It seems that adenosine and its A<sub>1</sub> and A<sub>2</sub> receptors exert different impact on the nociception under normal condition and after the exposure to environmental stress. According to Zarrindast et al. [19], A<sub>2</sub> receptor agonist NECA induced a potentiation of analgesia after exposure to swimming stress. Similarly to our present data, in a model of phasic pain reaction adenosine A<sub>1</sub> receptor agonist PIA decreased SIA, while a nonselective adenosine receptor blockade with theophylline augmented it [19].

Chronic treatment with adenosine antagonists activated variety long-term changes including up-regulation of the adenosine A<sub>1</sub> receptors, acetylcholine, serotonin receptors as well as benzodiazepine-binding sites [22]. The existence of symmetric cross-tolerance produced by caffeine and theophylline implies a common mechanism of action [23]. Chronic pretreatment with theophylline did not have inherent effect on the visceral pain and did not influence the antinociceptive effect of R-PIA. However, it limited the effect of R-PIA on SIA.

The present data showed that the endogenous adenosine was not able to alter RS-induced visceral antinociception, as theophylline did not show any effects on SIA. However, the selective adenosine A<sub>1</sub> receptor activation produced both an antinociception as well as abolished RS-induced antinociception, perhaps by different mechanisms.

**Acknowledgements:** This study was supported by COPERNICUS Program; contract No CIPA CT 94-0239.

### References

1. Calcagnetti DJ, Holtzman SG. Factors affecting restraint stress-induced potentiation of morphine analgesia. *Brain Res* 1990;537(1-2):157-162.
2. Tokuyama S, Takahashi M, Kaneto H. Further evidence for the participation of an alpha 2-adrenoceptor mediated mechanism in the production of forced swimming-stress induced analgesia in mice. *J Pharmacobiodyn* 1991;14(11):637-641.
3. Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB. Adenosine and brain ischemia. *Cerebrovasc Brain Metab Rev* 1992;4(4):346-369.
4. Dragunow M. Purinergic mechanisms in epilepsy. *Prog Neurobiol* 1988;31(2):85-108.
5. Sawynok J. Adenosine receptor targets for pain. *Neuroscience* 2016;338:1-18.
6. Maione S, de Novellis V, Cappellacci L, Palazzo E, Vita D, Luongo L, Stella L, Franchetti P, Marabese I, Rossi F, Grifantini M. The antinociceptive effect of 2-chloro-2'-C-methyl-N6-cyclopentyladenosine (2'-Me-CCPA), a highly selective adenosine A1 receptor agonist, in the rat. *Pain* 2007;131(3):281-292.
7. Fredholm BB. Adenosine receptors as drug targets. *Exp Cell Res* 2010;316(8):1284-8.

8. Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM, Reppert SM. Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. *Mol Brain Res* 1992;14:186-195.
9. Abo-Salem OM, Hayallah AM, Bilkei-Gorzo A, Filipek B, Zimmer A, Müller CE. Antinociceptive effects of novel A2B adenosine receptor antagonists. *J Pharmacol Exp Ther* 2004;308(1):358-66.
10. Luongo L, Guida F, Imperatore R, Napolitano F, Gatta L, Cristino L, Giordano C, Siniscalco D, Di Marzo V, Bellini G, Petrelli R, Cappellacci L, Usiello A, de Novellis V, Rossi F, Maione S. The A1 adenosine receptor as a new player in microglia physiology. *Glia* 2014;62(1):122-32.
11. Olah ME, Stiles GL. Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu Rev Pharmacol Toxicol* 1995;35:581-606.
12. Pechlivanova DM, Georgiev VP. Interaction of angiotensin II and adenosine A1 and A2A receptor ligands on the writhing test in mice. *Pharmacol Biochem Behav* 2002;72(1-2):23-28.
13. Segerdahl M, Karelav A. Experimentally induced ischaemic pain in healthy humans is attenuated by the adenosine receptor antagonist theophylline. *Acta Physiol Scand* 2004;180(3):301-306.
14. Ahlijanian MK, Takemori AE. Effects of (-)-N6-(R-phenylisopropyl)-adenosine (PIA) and caffeine on nociception and morphine-induced analgesia, tolerance and dependence in mice. *Eur J Pharmacol* 1985;112(2):171-179.
15. Zarrindast MR, Sabetkasai M, Khakpour S. Effects of drugs active at adenosine receptors on stress-induced analgesia in mice. *Arch Int Pharmacodyn Ther* 1993;325: 51-60.
16. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother* 1968;32(2):295-310.
17. Sawynok J, Sweeney MI. The role of purines in nociception. *Neuroscience* 1989;32(3):557-569.
18. Yang SW, Zhang ZH, Chen JY, Xie YF, Qiao JT, Dafny N. Morphine and norepinephrine-induced antinociception at the spinal level is mediated by adenosine. *Neuroreport* 1994;5(12):1441-1444.
19. Pechlivanova DM, Georgiev VP. Effects of single and long-term theophylline treatment on the threshold of mechanical nociception: contribution of adenosine A1 and alpha2-adrenoceptors. *Methods Find Exp Clin Pharmacol* 2005;27(9):659-664.
20. Nakagawasaki O, Tadano T, Tan No K, Niijima F, Sakurada S, Endo Y, Kisara K. Changes in beta-endorphin and stress-induced analgesia in mice after exposure to forced walking stress. *Methods Find Exp Clin Pharmacol* 1999;21(7):471-476.
21. Woolfolk DR, Holtzman SG. Restraint stress potentiates analgesia induced by 5'-N-ethylcarboxamido adenosine: comparison with morphine. *Eur J Pharmacol* 1993;239(1-3):177-182.
22. Traversa U, Rosati AM, Florio C, Vertua R. Effects of chronic administration of adenosine antagonists on adenosine A1 and A2a receptors in mouse brain. *In Vivo* 1994;8(6):1073-1078.
23. Finn IB, Holtzman SG. Tolerance and cross-tolerance to theophylline-induced stimulation of locomotor activity in rats. *Life Sci* 1988;42(24):2475-82.

**Figures Caption**

Fig. 1. Effects of a selective adenosine A<sub>1</sub> receptor agonist R-PIA (0.5 mg/kg), injected intraperitoneally (IP) on the visceral nociception (number of writhes for 30 minutes, after the IP injection of the irritant 1 % acetic acid) in control and restraint-stressed mice. \* p<0.05 vs saline group, # p<0.05 vs stressed controls, <sup>o</sup> p<0.05 vs R-PIA.

Effects of a selective adenosine A<sub>1</sub> receptor agonist R-PIA

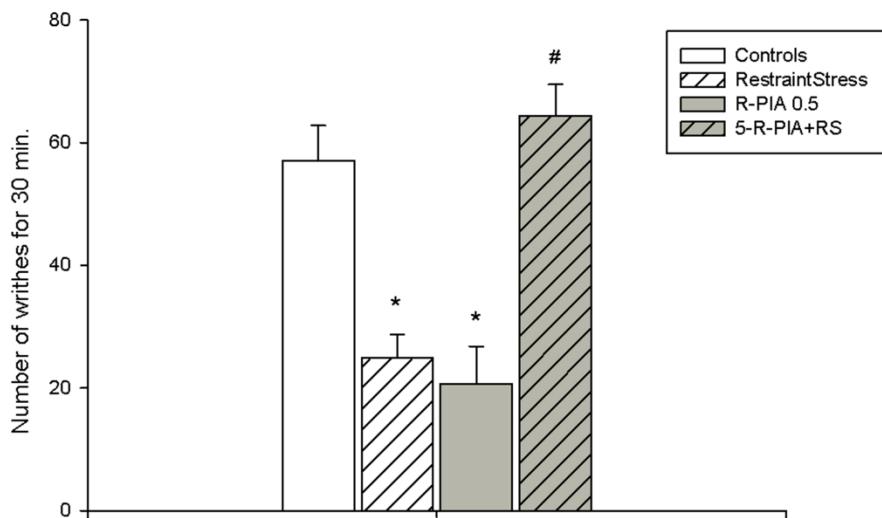


Fig. 2. Effects of a non-selective adenosine receptor antagonist theophylline (75 mg/kg), injected intraperitoneally (IP) at a single or chronic scheme of treatment on the visceral nociception (number of writhes for 30 minutes, after the IP injection of the irritant 1 % acetic acid) in control and restraint-stressed mice. \* p<0.05 vs saline group.

Effects of a nonselective adenosine receptor antagonist theophylline

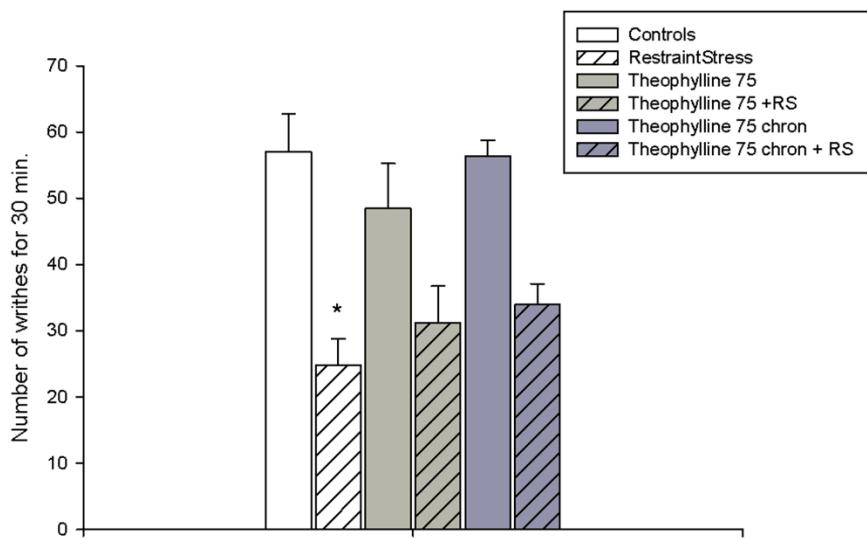
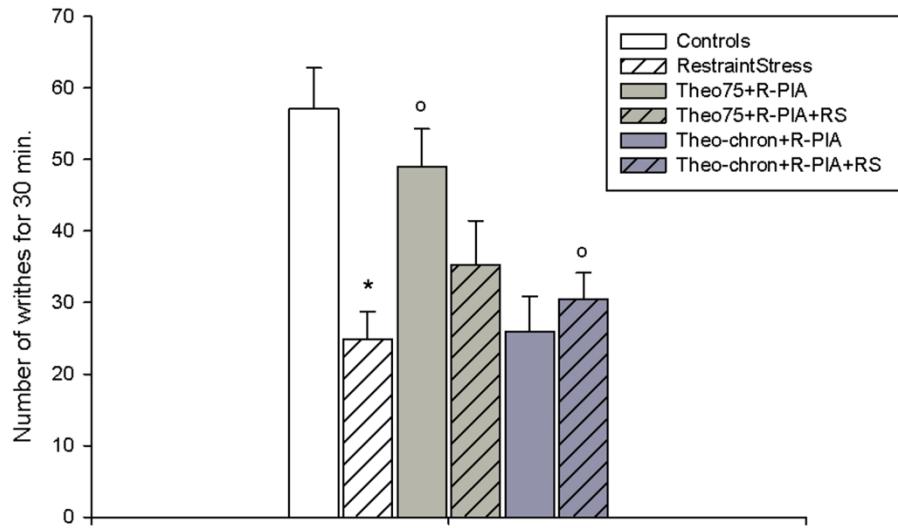


Fig. 3 Effects of a selective adenosine A1 receptor agonist R-PIA (0.5 mg/kg), injected intraperitoneally (IP) on the background of the single and chronic treatment with theophylline (75 mg/kg) on the visceral nociception (number of writhes for 30 minutes, after the IP injection of the irritant 1 % acetic acid) in control and restraint-stressed mice. \* p<0.05 vs saline group, <sup>o</sup> p<0.05 vs R-PIA.

Effects of R-PIA on the background of the theophylline (single and chronic)



**Corresponding author:**

Assoc. Prof. Daniela M. Pechlivanova  
Bulgarian Academy of Sciences  
Institute of Neurobiology  
Acad. G. Bonchev Str., Bl.23  
1113 Sofia, Bulgaria  
Tel.: 359-2-9792151  
Fax: 359-2-8719109  
E-mail: [pechlivanova@yahoo.com](mailto:pechlivanova@yahoo.com)