Adenosine receptor mediates nicotine-induced antinociception in formalin test

Homayoun Homayounfar[a], Nida Jamali-Raeufy[a], Mousa Sahebgharani[b], Mohammad-Reza Zarrindast[b],*

[a] *Department of Physiology, Iran University of Medical Science, Iran*
[b] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

Abstract

In this study, the effect of adenosine receptor agents on nicotine induced antinociception, in formalin test, has been investigated. Intraperitoneal (i.p.) administration of different doses of nicotine (0.1, 1, 10 and 100 $\mu g\,kg^{-1}$) induced a dose-dependent antinociception in mice, in the both first and second phases of the test. Adenosine receptor antagonist, theophylline (5, 10, 20 and 80 $mg\,kg^{-1}$, i.p.) also induced antinociception in the both phases, while a dose of the drug (40 $mg\,kg^{-1}$, i.p.) did not induce any response. Theophylline reduced antinociception induced by nicotine in both phases of formalin test. The A_2 receptor agonist, $5'$-N-ethylcarboxamide adenosine (NECA; 1 and 5 $\mu g\,kg^{-1}$, i.p.) also produced antinociception, which was reversed with different doses of theophylline (5, 10, 20 and 40 $mg\,kg^{-1}$, i.p.). But administration of the adenosine receptor agonist, NECA did not potentiate the response of nicotine. It is concluded that adenosine system may be involved in modulation of antinociception induced by nicotine.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Nicotine; NECA; Theophylline; Formalin test; Mice

1. Introduction

Adenosine is a neurotransmitter which, has generally inhibitory effect on nervous system [1], so that, adenosine receptor activation inhibits neural activity in many areas along the neuroaxis [2]. Moreover, it is one of the several endogenous compounds that may have a role in nociceptive information [3], and contributes to antinociception induced by oipioids, noradrenaline, 5-hydroxytryptamine, tricyclic antidepressants and transcutaneous electrical nerve stimulation [4]. Adenosine functions through at least three subtypes of adenosine receptor: A_1, A_2 and A_3 [5,6]. These receptor sites have been pharmacologically characterized by use of adenosine agonists and antagonists [7]. Adenosine has complex effects on pain transmission at peripheral and spinal sites, due to different subtypes of adenosine receptors.

In fact, there is a controversy on the role of A_1 adenosine receptors in antinociception. Some of studies confirm the antinociceptive effect of the receptors [3,8–12], while the others show the nociceptive response induced by the A_1 adenosine receptors [13–15]. Meanwhile, it has been suggested that activation of the peripheral A_1 adenosine receptors produce pronociceptive and pain enhancing effect [4,16].

Adenosine has recently been proposed to be a significant anti-inflammatory autacoid released peripherally under conditions of inflammation [17,18]. It seems that the A_2 receptor involves in the anti-inflammatory effect [18,19]. Within the spinal cord, activation of the both A_1 and A_2 produce antinociception. Antinociceptive actions of adenosine and adenosine analogs have been shown in a wide range of tests [4]. Adenosine receptor agonists have been proved to be more

* Corresponding author. Tel.: +98 21 6112801; fax: +98 21 6402569.
  E-mail address: zarinmr@ams.ac.ir (M.-R. Zarrindast).

potent in reducing hyperalgesia and allodynia than normal acute pain conditions [20,21].

The involvement of adenosine in antinociception and antiallodynia induced by opioids has also been demonstrated [22–24], and release of adenosine in the spinal cord contributes to the spinal efficacy of opioids [4].

Furthermore, nicotine, the psychoactive component of tobacco products, is widely consumed by humans [25–28]. The drug exhibits several pharmacological actions in the central and peripheral nervous systems and releases a number of neurotransmitters [29–32]. This drug is also able to activate endogenous opioid system(s) [33]. Acute nicotinic receptor stimulation activates enkephalin and beta-endorphin [34–38] release and biosynthesis in discrete brain nuclei and peripheral tissues. While, there are other reports indicating that chronic administration of nicotine reduces met-enkelphalin and beta-endorphin [37,39,40]. Moreover, the drug has been shown to induce antinociception in different tests [41–47]. The aim of this study was to investigate the effect of adenosine receptor agonist and antagonists on the antinociception induced by nicotine in mice.

2. Materials and methods

2.1. Animals

Male NMRI mice (20–30 g) were used in these experiments. They were kept 10 per cage (45 cm × 30 cm × 15 cm) at an environmental temperature of $23 \pm 1\,^\circ$C on a 12-h light–dark cycle. The animals had free access to food and water, except during the time of experiments. Each animal was used once only and was euthanized immediately after the experiment. The study was carried out according to institutional guideline for animal care and use.

2.2. Drugs

The following drugs (−)-nicotine base, adenosine agonist, 5′-(N-ethyl) carboxamido adenosine (NECA) and theophylline were purchased from Sigma–Aldrich, UK. Nicotine solutions were prepared in saline and the pH adjusted to 7.2 ± 0.1 with a small amount of NaOH and other drugs were dissolved in saline. All the drugs were injected intraperitoneally (i.p.) in a volume of $10\,\text{ml}\,\text{kg}^{-1}$.

2.3. Drug treatment

The animals were treated as follows: groups 1 and 2 received different doses of nicotine (0.1, 1, 10 and $100\,\mu\text{g}\,\text{kg}^{-1}$, i.p.) or theophylline (5, 10, 20, 40 and $80\,\text{mg}\,\text{kg}^{-1}$, i.p.), respectively and antinociception was assessed as described in Section 2.4. Group 3 received different doses of theophylline (5, 10 and $20\,\text{mg}\,\text{kg}^{-1}$, i.p.) in the presence or absence of lower dose of nicotine ($1\,\mu\text{g}\,\text{kg}^{-1}$, i.p.). Group 4 received different doses of theophylline (5, 10, 20 and $40\,\text{mg}\,\text{kg}^{-1}$, i.p.) in the presence or absence of higher dose of nicotine ($100\,\mu\text{g}\,\text{kg}^{-1}$, i.p.). Group 5 received different doses of nicotine (1, 5 and $10\,\mu\text{g}\,\text{kg}^{-1}$, i.p.) alone or nicotine plus NECA (1 and $5\,\mu\text{g}\,\text{kg}^{-1}$, i.p.). In all groups antinociception was assessed after nicotine injection.

2.4. Antinociception recording

Animals were allowed to acclimatize for 30 min before formalin injection. Twenty-five microliters of formalin (2.5%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a microsyringe with a 26-gauge needle. Immediately after formalin injection, animals were placed individually in a glass cylinder (20 cm wide, 25 cm long) on a flat glass floor and a mirror was arranged in a 45° angle under the cylinder to allow clear observation of the paws of the animals [48].

Pain response was recorded immediately after formalin injection for a period of 50 min. The total time (s) spent licking the injected paw during periods of 0–5 min (first phase) and 15–50 min (second phase) after formalin injection were measured as an indicator of pain.

2.5. Statistical analysis

One-way and two-way ANOVAs followed by Newman–Keuls test, were used for analysis of the data. Differences between means were considered statistically significant if $P < 0.05$. Each point is the mean ± S.E.M. of eight mice.

3. Results

3.1. Effect of nicotine or theophylline in formalin test

Fig. 1 indicates antinociception induced by nicotine in formalin test. One-way ANOVA showed that intraperitoneal injection of mice with different doses nicotine (0.1, 1, 10 and $100\,\mu\text{g}\,\text{kg}^{-1}$, i.p.) induced antinociception in the first [$F(4, 35) = 38.6$, $P < 0.0001$] (Fig. 1A) and second phases [$F(4, 35) = 63.6$, $P < 0.0001$] (Fig. 1B) of the test. The response of nicotine was maximum with $100\,\mu\text{g}\,\text{kg}^{-1}$ of the drug.

Fig. 2 indicates the response of theophylline in formalin test. One-way ANOVA indicated that administration of different doses of theophylline (5, 10, 20 and $80\,\text{mg}\,\text{kg}^{-1}$, i.p.) to mice induced antinociception in the first [$F(5, 42) = 9.6$, $P < 0.0001$] (Fig. 2A) and second [$F(5, 42) = 81.6$, $P < 0.0001$] (Fig. 2B) phases of formalin test. However, increasing of the drug doses decreased the response of drug. The drug in dose of $40\,\text{mg}\,\text{kg}^{-1}$, did not induce antinociception. However, the dose of $80\,\text{mg}\,\text{kg}^{-1}$, showed antinociception.

3.2. Effect of adenosine receptor agonist or antagonist on nicotine-induced antinociception in formalin test

Fig. 3 indicates effect of theophylline in the presence or absence of lower dose of nicotine. Two-way ANOVA showed
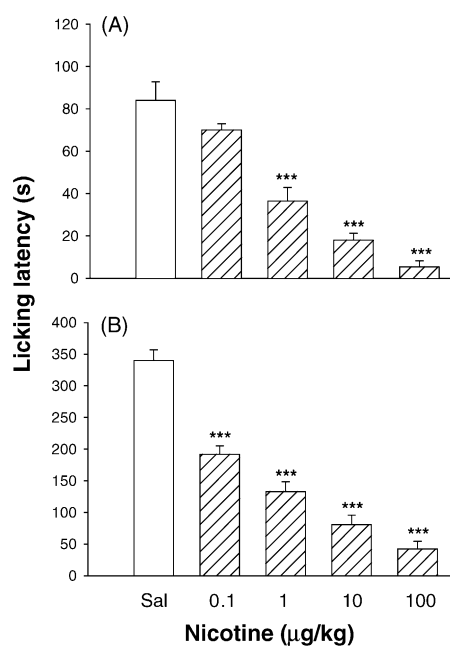
Fig. 1. Antinociceptive effect of nicotine in the formalin test. Mice were injected intraperitoneally (i.p.) either with saline (sal, 10 ml kg^{-1}) or different doses of nicotine (0.1, 1, 10 and 100 μg kg^{-1}) 15 min before formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. ***$P < 0.001$ different from respective saline control group.

that combination of theophylline (5, 10 and 20 mg kg^{-1}, i.p.) and lower dose of nicotine (1 μg kg^{-1}, i.p.) reduced nicotine response with interactions in the first [$F(3, 56) = 150.4$, $P < 0.0001$] (Fig. 3A) and second phase [$F(3, 56) = 107.0$, $P < 0.0001$] (Fig. 3B) of formalin test. Post hoc analysis also showed that the drugs induced antinociception in the both phases of the test.

Fig. 4 indicates effect of theophylline in the presence or absence of higher dose of nicotine. Two-way ANOVA showed that combination of theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) and higher dose of nicotine (100 μg kg^{-1}, i.p.) reduced nicotine response with interactions in the first [$F(5, 84) = 85.7$, $P < 0.0001$] (Fig. 4A) and second phase [$F(5, 84) = 193.6$, $P < 0.0001$] of formalin test (Fig. 4B). Post-hoc analysis also showed that the drugs induced antinociception in the both phases of the test.

Fig. 5A shows the antinociception induced by different doses of nicotine in the presence or absence of NECA in the first phase of formalin test. Two-way ANOVA indicated that combination of nicotine (1, 10 and 100 μg kg^{-1}, i.p.) with NECA (1 μg kg^{-1}, i.p.) [$F(3, 56) = 40.3$, $P < 0.0001$] and also nicotine with NECA 5 μg kg^{-1} [$F(3, 56) = 39.3$, $P < 0.0001$] induced interactions. Post hoc analysis also showed that NECA did not potentiate nicotine response in first phase of the test.
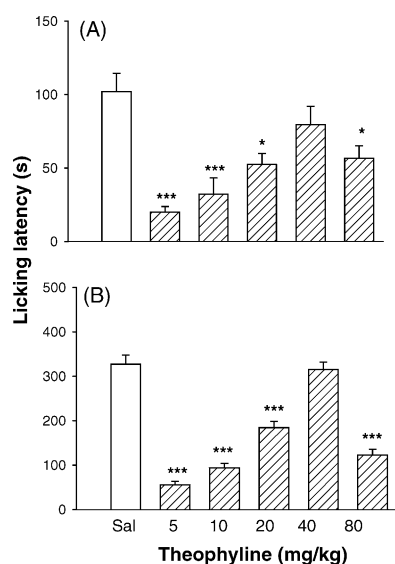

Fig. 2. Effect of theophylline in the formalin test. Animals were administered either saline (sal, 10 ml kg^{-1}) or different doses of theophylline (5, 10, 20, 40 and 80 mg kg^{-1}, i.p.) 60 min before formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. *$P < 0.05$, ***$P < 0.001$ different from respective saline control group.
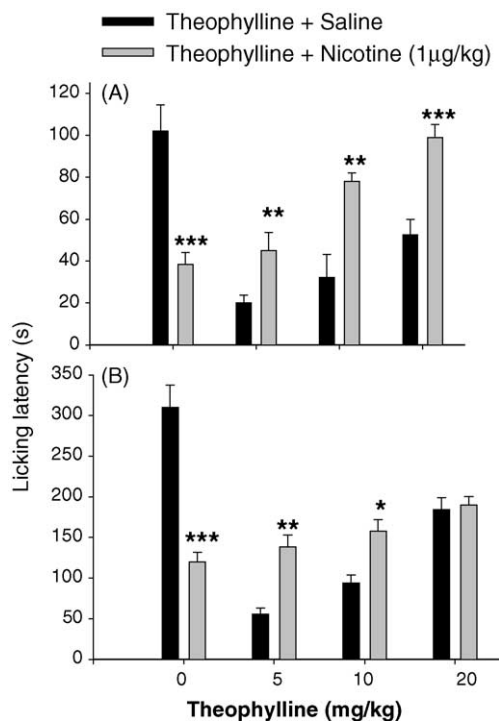

Fig. 3. Effect of theophylline in the presence or absence of lower dose of nicotine in the formalin test. Animals were administered either theophylline (5, 10 and 20 mg kg^{-1}, i.p.) 60 min before formalin injection, or theophylline plus nicotine (1 μg kg^{-1}, i.p.). Nicotine was administered 15 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ different from respective saline control group.
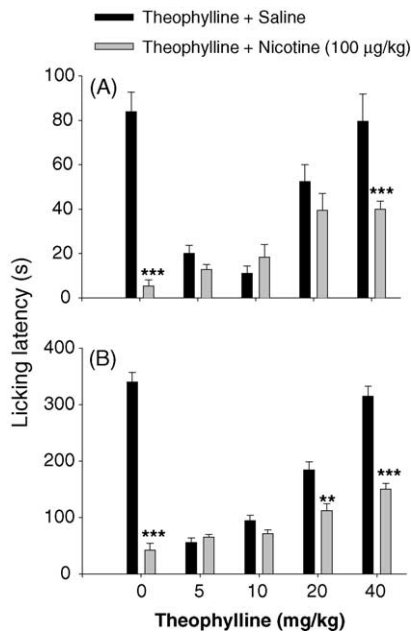
Fig. 4. Effect of theophylline in the presence or absence of higher dose of nicotine in the formalin test. Animals were administered either theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) 60 min before formalin injection, or theophylline plus nicotine (100 µg kg^{-1}, i.p.). Nicotine was administered 15 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective saline control group.
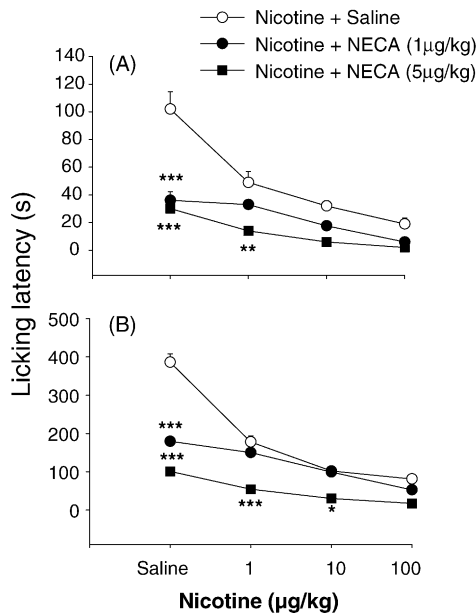

Fig. 5. Effect of nicotine in the presence or absence of 5'-N-ethylcarboxamide adenosine (NECA) in the formalin test. Animals were administered either nicotine (1, 10 and 100 µg kg^{-1}, i.p.) 15 min before formalin injection or nicotine plus NECA (1 and 5 µg kg^{-1}, i.p.). NECA was administered 30 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ different from respective saline control group.

Fig. 5B shows the antinociception induced by nicotine in the presence or absence of NECA in the second phase of formalin test. Two-way ANOVA indicated that combination of nicotine (1, 10 and 100 µg kg^{-1}, i.p.) and NECA 1 µg kg^{-1} [$F(3, 56) = 48.8$, $P < 0.0001$] and NECA 5 µg kg^{-1} [$F(3, 56) = 66.9$, $P < 0.0001$] induced interactions. Further analysis showed that NECA did not potentiate the response of nicotine in the second phase of the test. NECA in doses higher than 5 µg kg^{-1} (10, 50 and 100 µg kg^{-1}, i.p.) induced antinociception, which a part of the response may be due to sedation.

3.3. Effect of theophylline on adenosine-induced antinociception in formalin test

Antinociception induced by different doses of theophylline (5, 10, 20 and 50 mg kg^{-1}, i.p.) in the presence or absence of NECA 5 µg kg^{-1} is shown in Fig. 6. Two-way ANOVA showed that combination of theophylline with NECA induced interaction in the first phase (Fig. 6A) [$F(3, 56) = 48.8$, $P < 0.0001$] and second phase (Fig. 6B) [$F(3, 56) = 48.8$, $P < 0.0001$] of the formalin test. Further analysis
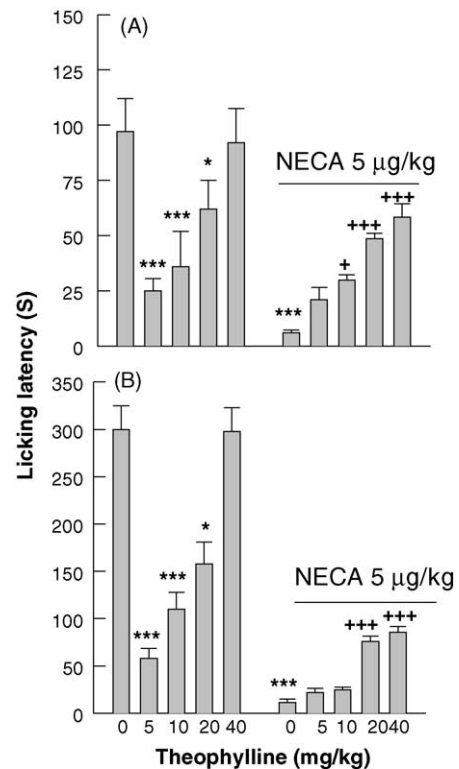

Fig. 6. Effect of theophylline in the presence or absence of 5'-N-ethylcarboxamide adenosine (NECA) in the formalin test. Animals were administered either theophylline (5, 10, 20 and 40 mg kg^{-1}, i.p.) 60 min before formalin injection or theophylline plus NECA (5 µg kg^{-1}, i.p.). NECA was administered 30 min prior to formalin injection. Antinociception during 0–5 min (panel A; first phase) and 15–50 min (panel B; second phase) after formalin injection was recorded. Each point is the mean ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ different from respective saline control group. $^{+}P < 0.05$, $^{+++}P < 0.001$ different from respective NECA control group.

showed that theophylline reversed the antinociceptive effect of NECA.

4. Discussion

The formalin test is a model of injury-produced pain, which was introduced by Dubuisson and Dennis [49]. It measures the response to a long-lasting nociceptive stimulus; resemble clinical pain. The test produced a distinct biphasic response. The early response (first phase) was recorded during the 5 min after formalin injection, and the late response (second phase) recorded 20–50 min after formalin injection. It has been reported that the action of analgesics differs in the early phase and late phase [50–52].

The present data showed that nicotine induced a dose-dependent antinociception in the first and second phases of formalin test. This is in agreement with previous reports that nicotine induces antinociception (see Section 1). Interaction between nicotinic and opioid systems has been observed [33,36]. The antinociceptive effect of nicotine is shown to be mediated through cholinergic [42], opioid receptor [53] and GABA-A receptor mechanisms [54]. Bernardini et al. [55] showed that nicotine can weakly excite C-nociceptors, while muscarinic receptor desensitization leads to antinociception. There are also some reports indicating interaction of nicotinic and adenosine receptors [56,57]. Nicotine can cause the release of endogenous opioid peptides [33,58,59] and these peptides are able to release adenosine [60–63].

The present study also showed that theophylline, an adenosine antagonist and also a phosphodiesterase inhibitor [64], induced antinociception. However, the drug showed a dual effect. Antinociception decreased by increasing the dose of the drug to $40 \, \text{mg} \, \text{kg}^{-1}$ and increased again in $80 \, \text{mg} \, \text{kg}^{-1}$. This is in agreement with the data showing that increase in doses of methylxanthines decreases antinociception [65,66]. As well as, it has already been shown that theophylline exerts dual effect in other experiments such as neurotransmitter release [67] or effect on morphine analgesic effect [68].

Theophylline blocks both A_1 and A_2 adenosine receptors, it can be postulated that antinociceptive effect of theophylline and decreasing its response may be due to blockade of the two different adenosine receptor subtypes. There is controversial results on role of the A_1 receptors (see Section 1). It has been reported that activation of A_1 receptors produce pronociceptive and pain enhancing effect, while A_2 receptor induces antinociceptive response. Therefore, the possibility may exist that blockade of A_1 receptor by the lower doses of theophylline induces antinociception, while the increase in doses of the drug which may block A_2 receptor, showed less response. This hypothesis is supported by our data indicating that NECA, which may have more affinity on A_2 adenosine receptor [69–71] induced antinociception, which is also in agreement by other results in this respect [72,73]. Therefore the antinociceptive effect of NECA was reversed by higher doses of theophylline (20 and $40 \, \text{mg} \, \text{kg}^{-1}$) in the present study.

In the present study, the effects of nicotine in the presence or absence of theophylline or adenosine receptor agonist, NECA have been investigated in the formalin test. However, adenosine mechanism may be involved in the nicotine response, our present data showed that NECA did not potentiate the response of nicotine and the additive effect may be involved in the response of combination of the two drugs.

On the other hand, combination of nicotine with theophylline elicits lower antinociceptive effect, which can support adenosine receptor mechanism in the antinociceptive response of nicotine. This data even further supports the hypothesis that blockade of the A_2 receptors by theophylline decreases antinociception. In addition to inhibition of phosphodiesterase and $5'$-nucleotide, methylxanthines are reported to have a variety of actions unrelated to their antagonism of adenosine receptors, including alterations in intracellular Ca^{2+} concentrations and modulation of GABA or noradrenergic transmission [74,75]. Whether, these mechanisms are involved in the present theophylline effects, it should be examined. Overall, it is concluded that adenosine receptor mechanism may be involved in modulation of nicotine-induced antinociception.

Acknowledgement

References

[1] Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001;24:31–55.

[2] Stone TW. Receptors for adenosine and adenine nucleotides. Gen Pharmacol 1991;22:25–31.

[3] Nakamura I, Ohta Y, Kemmotsu O. Characterization of adenosine receptors mediating spinal sensory transmission related to nociceptive information in the rat. Anesthesiology 1997;87:577–84.

[4] Sawynok J. Adenosine receptor activation and nociception. Eur J Pharmacol 1998;317:1–11.

[5] Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 2001;53:527–52.

[6] Fredholm BB, Abbracchio MP, Burnstock G, Dubyak GR, Harden TK, Jacobson KA, et al. Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol Sci 1997;18:79–82.

[7] Palmer TM, Stiles GL. Adenosine receptors. Neuropharmacology 1995;34:683–94.

[8] Giffin NJ, Kowacs F, Libri V, Williams P, Goadsby PJ, Kaube H. Effect of the adenosine A_1 receptor agonist GR79236 on trigeminal nociception with blink reflex recordings in healthy human subjects. Cephalalgia 2003;23(4):287–92.

[9] Malhotra J, Chaudhary G, Gupta Y. Dopaminergic involvement in adenosine A_1 receptor-mediated antinociception in the tail flick latency model in mice. Methods Find Exp Clin Pharmacol 2000;22(1):37–41.

[10] Sawynok J, Sweeney MI, White TD. Classification of adenosine receptors mediating antinociception in the rat spinal cord. Br J Pharmacol 1986;88(4):923–30.

[11] Keil II GJ, DeLander GE. Adenosine kinase and adenosine deaminase inhibition modulate spinal adenosine-and opioid agonist-induced antinociception in mice. Eur J Pharmacol 1994;271(1):37–46.

[12] Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Gimenez-Llort L, et al. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. Proc Natl Acad Sci USA 2001;98(16):9407–12.

[13] 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.

[14] Sawynok J, Reid A. Neurotoxin-induced lesions to central serotonergic, noradrenergic and dopaminergic systems modify caffeine-induced antinociception in the formalin test and locomotor stimulation in rats. J Pharmacol Exp Ther 1996;277(2):646–53.

[15] Ghelardini C, Galeotti N, Bartolini A. Caffeine induces central cholinergic analgesia. Naunyn Schmiedebergs Arch Pharmacol 1997;356(5):590–5.

[16] Dowd E, McQueen DS, Chessell IP, Humphrey PP. Adenosine A_1 receptor-mediated excitation of nociceptive afferents innervating the normal and arthritic rat knee joint. Br J Pharmacol 1998;125(6):1267–71.

[17] Krump E, Borgeat P, Adenosine. An endogenous inhibitor of arachidonic acid release and leukotriene biosynthesis in human neutrophils. Adv Exp Med Biol 1999;447:107–15.

[18] Sullivan GW. Adenosine A2A receptor agonists as anti-inflammatory agents. Curr Opin Invest Drugs 2003;4(11):1313–9.

[19] Sitkovsky MV. Use of the A(2A) adenosine receptor as a physiological immunosuppressor and to engineer inflammation in vivo. Biochem Pharmacol 2003;65(4):493–501.

[20] Yamamoto T, Yaksh TL. Spinal pharmacology of thermal hyperalgesia induced by incomplete ligation of sciatic nerve. Pharmacol Biochem Behav 1991;17:817–26.

[21] Lee YW, Yaksh TL. Pharmacology of spinal adenosine receptor, which mediates the antiallodynic action of intrathecal adenosine. J Pharmacol Exp Ther 1996;277:1618–42.

[22] Borghi V, Przewlocka B, Labuz D, Maj M, Ilona O, Pavone F. Formalin-induced pain and mu-opioid receptor density in brain and spinal cord are modulated by A_1 and A2A adenosine agonists in mice. Brain Res 2002;956:339–48.

[23] Bailey A, Ledent C, Kelly M, Hourani SM, Kitchen I. Changes in spinal delta and kappa opioid systems in mice deficient in the A2A receptor gene. J Neurosci 2002;22:9210–20.

[24] Lavand'homme PM, Eisenach JC. Exogenous and endogenous adenosine enhances the spinal antiallodynic effects of morphine in a rat model of neuropathic pain. Pain 1999;80:31–6.

[25] Sutherland G. Current approaches to the management of smoking cessation. Drugs 2002;62:53–61.

[26] Garrett BE, Rose CA, Henningfield JE. Tobacco addiction and pharmacological interventions. Expert Opin Pharmacother 2001;2:1545–55.

[27] Pulvirenti L, Diana M. Drug dependence as a disorder of neural plasticity: focus on dopamine and glutamate. Rev Neurosci 2001;12:141–58.

[28] Shields PG. Epidemiology of tobacco carcinogenesis. Curr Oncol Rep 2000;2:257–62.

[29] Miller DK, Harrod SB, Green TA, Wong MY, Bardo MT, Dwoskin LP. Lobeline attenuates locomotor stimulation induced by repeated nicotine administration in rats. Pharmacol Biochem Behav 2003;74:279–86.

[30] Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 2002;13:451–63.

[31] Yokotani K, Okada S, Nakamura K. Characterization of functional nicotinic acetylcholine receptors involved in catecholamine release from the isolated rat adrenal gland. Eur J Pharmacol 2002;446:83–7.

[32] Mihailescu S, Guzman-Marin R, DominguezMdel C, Drucker-Colin R. Mechanisms of nicotine actions on dorsal raphe serotoninergic neurons. Eur J Pharmacol 2002;452:77–82.

[33] Davenport KE, Houdi AA, Van Loon GR. Nicotine protects against μ-opioid receptor antagonism by β-funaltrexamine: evidence for nicotine-induced release of endogenous opioids in brain. Neurosci Lett 1990;113:40–6.

[34] Isola R, Duchemin AM, Tejwani GA, Neff NH, Hadjiconstantinou M. Glutamate receptors participate in the nicotine-induced changes of met-enkephalin in striatum. Brain Res 2000;878:72–8.

[35] Van Loon GR, Pierzchala K, Houdi AA. Nicotine-induced alterations in peripheral tissue concentrations of native and cryptic Met- and Leu-enkephalin. Neuropeptides 1991;19:35–41.

[36] Houdi AA, Pierzchala K, Marson L, Palkovits M, Van Loon GR. Nicotine-induced alteration in Tyr–Gly–Gly and Met-enkephalin in discrete brain nuclei reflects altered enkephalin neuron activity. Peptides 1991;12:161–6.

[37] Boyadjieva NI, Sarkar DK. The secretary response of hypothalamic beta-endorphin neurons to acute and chronic nicotine treatments and following nicotine withdrawal. Life Sci 1997;61:PL59–66.

[38] Jensen RA, Gilbert DG, Meliska CJ, Landrum TA, Szary AB. Characterization of a dose–response curve for nicotine-induced conditioned taste aversion in rats: relationship to elevation of plasma beta-endorphin concentration. Behav Neural Biol 1990;53:428–40.

[39] Wewers ME, Dhatt RK, Snively TA, Tejwani GA. The effect of chronic administration of nicotine on antinociception, opioid receptor binding and met-enkelphalin levels in rats. Brain Res 1999;822:107–13.

[40] Rasmussen DD. Effects of chronic nicotine treatment and withdrawal on hypothalamic proopiomelanocortin gene expression and neuroendocrine regulation. Psychoneuroendocrinology 1998;23:245–59.

[41] Zarrindast MR, Babaei-Nami A, Farzin D. Nicotine potentiates morphine antinociception: a possible cholinergic mechanism. Eur Neuropsychopharmacol 1996;6:127–33.

[42] Zarrindast MR, Pazouki M, Nassiri-Rad S. Involvement of cholinergic and opioid receptor mechanisms in nicotine induced antinociception. Pharmacol Toxicol 1997;81:209–13.

[43] Decker MW, Meyer MD, Sullivan JP. The therapeutic potential of nicotinic acetylcholine receptor agonists for pain control. Expert Opin Invest Drugs 2001;10:1819–30.

[44] Schmidt BL, Tambeli CH, Gear RW, Levine JD. Nicotine withdrawal hyperalgesia and opioid-mediated analgesia depend on nicotine receptors in nucleus accumbens. Neuroscience 2001;106:129–36.

[45] Mandillo S, Kanarek RB. Chronic sucrose intake enhances nicotine-induced antinociception in female but not male Long-Evans rats. Pharmacol Biochem Behav 2001;68:211–9.

[46] Damaj MI. The involvement of spinal Ca(2+)/calmodulin protein kinase II in nicotine-induced antinociception in mice. Eur J Pharmacol 2000;404:103–10.

[47] Decker MW, Rueter LE, Bitner RS. Nicotinic acetylcholine receptor agonists: a potential new class of analgesics. Curr Top Med Chem 2004;4(3):369–84.

[48] Leland NH, Barry DG. Changes of substance P-like immunoreactivity in the dorsal horn are associated with the plastic behavioral response to formalin stimulus. Brain Res 1990;537:287–92.

[49] Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977;4:161–74.

[50] Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.

[51] Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995;60:91–102.

[52] Jett MF, Michelson S. The formalin test in rat: validation of an automated system. Pain 1996;64:19–25.

[53] Berrendero F, Kieffer BL, Maldonado R. Attenuation of nicotine-induced antinociception, rewarding effects, and dependence in mu-opioid receptor knock-out mice. J Neurosci 2002;22(24):10935–40.

[54] Mui WC, Zbuzek VK, Wu WH. GABA(A) antagonist and nicotine-induced antinociception. Life Sci 1997;61(15):PL221–5.

[55] Bernardini N, Sauer SK, Haberberger R, Fischer MJ, Reeh PW. Excitatory nicotinic and desensitizing muscarinic (M2) effects on C-nociceptors in isolated rat skin. J Neurosci 2001;21(9):3295–302.

[56] Dar MS, Bowman ER, Li C. Intracerebellar nicotinic-cholinergic participation in the cerebellar adenosinergic modulation of ethanol-induced motor incoordination in mice. Brain Res 1994;644(1):117–27.

[57] Smits P, Eijsbouts A, Thien T. Nicotine enhances the circulatory effects of adenosine in human beings. Clin Pharmacol Ther 1989;46(3):272–8.

[58] El-Guebaly IZ. Nicotine and endogenous opioids: toward specific pharmacotherapy. Can J Psychiatry 1998;43(1):37–42.

[59] Pomerleau OF. Endogenous opioids and smoking: a review of progress and problems. Psychoneuroendocrinology 1998;23(2):115–30.

[60] Eisenach JC, Hood DD, Curry R, Sawynok J, Yaksh TL, Li X. Intrathecal but not intravenous opioids release adenosine from the spinal cord. J Pain 2004;5(1):64–8.

[61] Sandner-Kiesling A, Li X, Eisenach JC. Morphine-induced spinal release of adenosine is reduced in neuropathic rats. Anesthesiology 2001;95(6):1455–9.

[62] Cahill CM, White TD, Sawynok J. Substance P releases and augments the morphine-evoked release of adenosine from spinal cord. Brain Res 1997;760(1–2):294–7.

[63] Cahill CM, White TD, Sawynok J. Synergy between mu/delta-opioid receptors mediates adenosine release from spinal cord synaptosomes. Eur J Pharmacol 1996;298(1):45–9.

[64] Choi OH, Shamis MT, Padgett WL, Dally JW. Caffeine and theophylline analogues: correlation of behavioural effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci 1988;43:387–98.

[65] Zarrindast MR, Matinrokh H, Mojtahedzadeh-Ardebili P. Adenosine receptor agonists or antagonists alter antinociception, but did not show an interaction with imipramine-induced antinociception in the formalin test in mice. Eur Neuropsychopharmacol 2003;13(3):165–72.

[66] Keil II GJ, Delander GE. Altered sensory behaviors in mice following manipulation of endogenous spinal adenosine neurotransmission. Eur J Pharmacol 1996;312:7–14.

[67] Barry SR. Dual effects of theophylline on spontaneous transmitter release from frog motor nerve terminals. J Neurosci 1988;8(12):4427–33.

[68] Malec D, Michalska E. The effect of methylxanthines on morphine analgesia in mice and rats. Pol J Pharmacol Pharm 1988;40(3):223–32.

[69] Rubovszky B, Szentmiklosi AJ, Marian T, Cseppento A, Gesztelyi R, Szekely A, et al. Comparative pharmacological studies on the A$_2$ adenosine receptor agonist 5'-*n*-ethyl-carboxamidoadenosine and its F19 isotope labelled derivative. J Pharmacol Sci 2003;93(3):356–63.

[70] Heffner TG, Wiley JN, Williams AE, Bruns RF, Coughenour LL, Downs DA. Comparison of the behavioral effects of adenosine agonists and dopamine antagonists in mice. Psychopharmacology (Berlin) 1989;98(1):31–7.

[71] Bruns RF, Lu GH, Pugsley TA. Characterization of the A$_2$ adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. Mol Pharmacol 1986;29(4):331–46.

[72] Sawynok J, Reid A. Desipramine potentiates spinal antinociception by 5-hydroxytryptamine, morphine and adenosine. Pain 1992;50:113–8.

[73] Zhao Y, Zhang C, Kang Y, Qiao J, Dafny N. Endogenous adenosine involved in the mediation of spinal antinociception produced by stimulating locus coeruleus. Life Sci 1999;65:67–74.

[74] Nehlig A, Daval JL, Derby G. Caffeine and central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev 1992;17:139–70.

[75] Sawynok J, Yaksh TL. Caffeine as an analgesic adjuvant: a review of pharmacology and mechanisms of action. Pharmacol Rev 1993;45:43–85.