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Altered sensory behaviors in mice following manipulation of endogenous spinal adenosine neurotransmission

Gary J. Keil II *, Gary E. DeLander

College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

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Abstract

Adenosine or adenosine analogs injected intrathecally (i.t.) induce significant antinociception. Recent studies support the existence of an endogenous spinal system that can modulate nociceptive input by releasing adenosine. Inhibition of adenosine metabolism by administration of an adenosine kinase inhibitor, in the present study, decreased behavior induced by putative pain neurotransmitters providing additional support for an endogenous purinergic system. Conversely, administration of high doses of methylxanthines (i.t.), adenosine receptor antagonists, induced behavior similar to that induced by pain neurotransmitters. Methylxanthine (i.t.)-induced behavior was partially inhibited by antagonists of receptors for pain neurotransmitters. These observations are consistent with the hypothesis that an endogenous purinergic system tonically modulates nociceptive input involving a variety of chemical mediators. Preliminary studies also revealed methylxanthine-induced allodynia and suggested spinal purinergic systems may have a broader role in discriminating sensory input.

Keywords: Adenosine, pharmacology; Antinociception; Allodynia; Spinal cord; (Mouse)

1. Introduction

Adenosine and adenosine analogs induce a wide variety of effects in the central and peripheral nervous systems. In general, adenosine receptor activation inhibits neuronal activity in many areas along the neuraxis (reviewed, Stone, 1991). Adenosine receptor agonists induce antinociception and a broad range of investigations suggest the spinal cord may be the primary site of adenosine-induced antinociception (reviewed, Sawynok and Sweeney, 1989; Salter et al., 1993).

Intrathecal (i.t.) administration of adenosine receptor agonists induces antinociception in several traditional behavioral assays for antinociception including tail flick and hot plate tests in mice (Post, 1984; DeLander and Hopkins, 1986, 1987) and rats (Jurna, 1984; Holmgren et al., 1986; Sawynok et al., 1986), and inhibit behavior induced by putative 'pain' neurotransmitters (DeLander and Wahl, 1988). Spinally administered adenosine analogs also induce antinociception in several less 'traditional' models including visceral nociception (Sosnowski et al., 1989), phase 2 of the formalin test (Malmberg and Yaksh, 1993), thermal hyperalgesia following nerve compression (Yamamoto and Yaksh, 1991) and allodynia induced by i.t. strychnine (Sosnowski et al., 1989) or prostaglandin $F_{2\alpha}$ (Minami et al., 1992). Finally, several studies by Sollevi and colleagues (Sollevi et al., 1995; Segerdahl et al., 1995; Belfrage et al., 1995) have demonstrated adenosine administration provides profound pain relief in human patients with pathological and experimental pains. Although the studies in humans did not specifically address the site of adenosine-mediated analgesia, the spinal cord may be involved.

Several lines of evidence support a role for endogenous spinal adenosine in the modulation of sensory input. Spinal localization of adenosine receptors (Geiger et al., 1984; Bruns et al., 1986; Choca et al., 1987, 1988), adenosine deaminase (Geiger and Nagy, 1986), nucleoside transporters (Geiger and Nagy, 1985) and adenosine-like immunoreactivity in the substantia gelatinosa (Braas et al., 1986) suggest adenosine neurotransmission is a component of spinal sensory processing. Our laboratory (Keil and DeLander, 1992, 1994, 1995) and others (Poon and

* Corresponding author. Present address: Division of Neurosciences, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. Tel.: (416) 813-5021; fax: (416) 813-7921; e-mail: g.keil@utoronto.ca

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Sawynok, 1995) have recently demonstrated manipulations which increase endogenous adenosine concentrations induce antinociception or significantly affect adenosine- and opioid-mediated antinociception (Keil and DeLander, 1994, 1995). These observations of enhanced effects following pharmacologic manipulation of endogenous adenosine in assays for antinociception are supported by similar experiments and results in seizure models (Zhang et al., 1993).

In contrast to the antinociceptive effects by exogenous adenosine analogs or manipulation of endogenous adenosine, administration of adenosine receptor antagonists is reported to facilitate nociceptive neurotransmission. Systemic administration of adenosine receptor antagonists facilitates a supraspinally integrated nociceptive threshold in awake rats (Paalzow and Paalzow, 1973; Paalzow, 1994). Spinal administration of adenosine receptor antagonists induces thermal hyperalgesia under certain experimental conditions (Jurna, 1984; Sawynok et al., 1986) and may induce nociceptive behavior (Nagaoka et al., 1993). Altered nociceptive thresholds following inhibition of adenosine receptors suggest these treatments may disinhibit excitatory processes or perhaps induce a 'miscoding' of non-nociceptive sensory input.

Our laboratory has previously proposed (Keil and DeLander, 1994, 1995) an endogenous adenosine 'tone' may modulate sensory input at spinal sites. The present experiments were carried out to specifically examine effects of pharmacologic manipulations that increase endogenous adenosine upon behavior induced by putative pain neurotransmitters and to characterize similar behavior induced by i.t. administration of adenosine receptor antagonists. We observed that behavior induced by either putative pain neurotransmitters and adenosine receptor antagonists was inhibited by endogenous adenosine or administration of adenosine agonists, respectively. In addition, adenosine receptor antagonist-induced behavior was also inhibited by selected antagonists for putative pain neurotransmitter receptors. Finally, preliminary experiments also revealed development of a delayed tactile allodynia following i.t. administration of adenosine receptor antagonists.

2. Materials and methods

2.1. Animals

Male, Swiss-Webster mice (Simonsen, Gilroy, CA) weighing 20–30 g were used for all experiments. Mice were housed in groups of 5 in a temperature-controlled room with a 12 h light/dark schedule. Food and water were available ad libitum until time of experiments. Animals were allowed to acclimate to the testing room for at least 1 h before each experiment. All studies were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee of Oregon State University (Corvallis, OR, USA).

2.2. Assays for nociception / antinociception

Caudally directed biting and scratching behavior induced by i.t. injection of excitatory amino acids or substance P was quantitated as a measure of the degree of nociception induced by these putative pain neurotransmitters. Briefly, the excitatory amino acids, *N*-methyl-D-aspartate (NMDA) (250 pmol), (*S*) α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (12.5 pmol) or kainic acid (60 pmol), or the tachykinin, substance P (6 pmol), were injected (i.t.) and the number of bites and scratches to the hind quarters and tail were quantitated during a 1 min observation period beginning immediately after placement of the animal in the observation chamber. Doses of excitatory amino acids and substance P were chosen to induce between 80 and 100 behaviors during the testing period (Aanonsen and Wilcox, 1987; DeLander and Wahl, 1988, 1989, 1991).

The antinociceptive effect of endogenous adenosine to inhibit biting and scratching behavior was investigated by assessing behavior following treatments with inhibitors of adenosine kinase or adenosine deaminase. An adenosine kinase inhibitor, 5′-amino 5′-deoxyadenosine, or an adenosine deaminase inhibitor, 2′-deoxycoformycin, was administered i.t. and behavior induced by NMDA, AMPA, kainic acid and substance P was quantitated as described above.

2.3. Nociceptive effects of adenosine receptor antagonists

2.3.1. Biting and scratching

Nociceptive behavior induced by i.t. administration of adenosine receptor antagonists was evaluated because previous studies (Paalzow and Paalzow, 1973; Jurna, 1984; Sawynok et al., 1986; Nagaoka et al., 1993; Keil and DeLander, 1994; Paalzow, 1994) suggest an inhibitory purinergic 'tone' may exist. Nonselective adenosine receptor antagonists, theophylline and 8-*p*-sulphophenyl theophylline (8-*p*SPtheophylline), were administered i.t. and caudally directed biting and scratching evaluated. Unlike behavior induced by the excitatory amino acids or substance P, adenosine receptor antagonist-induced effects were persistent, lasting approximately 10 min. Biting and scratching behavior induced by theophylline and 8-*p*SPtheophylline was quantitated over the entire duration of action.

To evaluate the possible disinhibition of spinal excitatory amino acid or NK_1 (tachykinin) receptors following spinal adenosine receptor antagonism, caudally directed biting and scratching behavior induced by theophylline was evaluated for 10 min following coadministration with the excitatory amino acid receptor antagonists, D-2-amino-5-phosphonovaleric acid (APV), (±)-5-methyl-10,11-dihydro-5*H*-dibenzo(a,d)cycloheptene-5,10-imine maleate (MK-801), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or 4-hydroxyquinoline-2-carboxylic acid (kynurenate), or

the NK_1 receptor antagonist, [D-Arg[1],D-Trp[7,9],Leu[11]]substance P (spantide).

2.3.2. Allodynia

Preliminary investigations were also conducted to examine potential allodynic effects of spinal adenosine receptor antagonists. Adenosine receptor antagonists or vehicle were administered (i.t.) and the mice immediately placed in a clear plexiglass observation chamber (12" × 12"). Quantification of the magnitude of behaviors was carried out according to the method of Yaksh and Harty (1988). Briefly, behavioral response to touch was assessed at various time points by light stroking the hind quarters (for approximately 5 s) with a smooth glass rod. The allodynic response [also referred to as touch-evoked agitation] was ranked as follows: 0, no response; 1, mild squeaking with attempts to move away from the stroking probe; or 2, vigorous squeaking evoked by the stroking probe, biting at the probe, strong efforts to escape and touch-evoked agitation. Each mouse was tested every 5 min for 20 min, then again at times 30, 45, 60 and 90 min. Control mice (vehicle injected) were similarly evaluated for behaviors at each time point. Allodynic responses were verified, but not quantified, by an independent observer.

2.4. Drugs and drug administration

All drugs were solubilized and injected i.t. (Hylden and Wilcox, 1980) in a total volume of 5 μl. For coadministration studies, compounds were mixed together in solution. For studies involving multiple i.t. injections, drugs or the corresponding vehicles were administered at the times indicated. Administration protocols were such that the peak effect of each compound occurred simultaneously. Neither drug vehicle nor drug administration protocols had significant effects on control values. All compounds were dissolved in normal saline except 8-*p*SPtheophylline (water), CNQX (1.25% 2-hydroxypropyl β-cyclodextrin) and kynurenate (0.1 M NaOH).

2.5. Statistics

Comparisons between groups following drug treatments were performed by one way analysis of variance or Kruskal-Wallis analysis of variance on ranks followed by appropriate post-hoc *t*-tests. Critical values that achieved $P < 0.05$ were considered statistically significant.

3. Results

5′-Amino-5′-deoxyadenosine (i.t.) pretreatment (10 min) dose dependently inhibited caudally directed biting and scratching behavior induced by kainic acid and substance P administered i.t. (Fig. 1). 5′-Amino-5′-deoxyadenosine (i.t.) partially inhibited biting and scratching induced by AMPA (maximal inhibition approximately 50%) but was

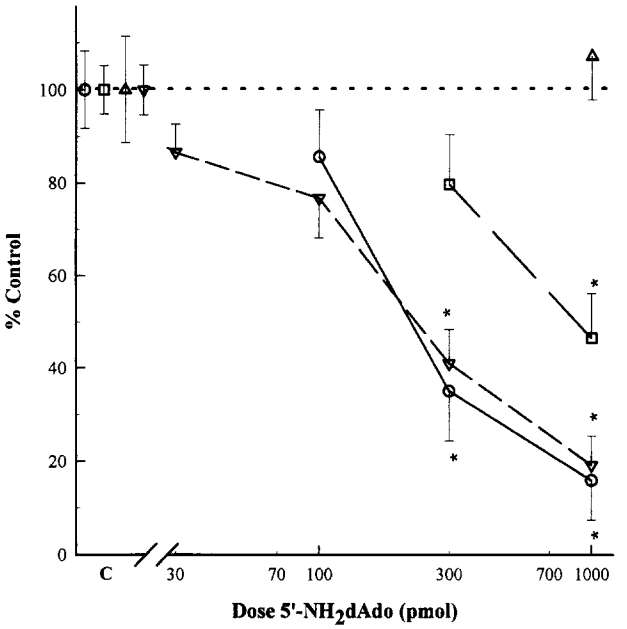

Fig. 1. Antinociceptive effects of the adenosine kinase inhibitor, 5′-amino 5′-deoxyadenosine, on behavior induced by excitatory amino acids or substance P. Graded doses of 5′-amino-5′-deoxyadenosine were coadministered (i.t.) with doses of NMDA (△; 250 pmol), AMPA (□; 12.5 pmol), kainic acid (○; 60 pmol), or substance P (▽; 6 pmol) each of which induced approximately 80–100 behaviors (100% Control (C) over the first min of testing (mean ± S.E.M.; $n = 7–10$). * Significant reductions in excitatory amino acid- or substance P-induced effects following 5′-amino 5′-deoxyadenosine i.t. coadministration ($P < 0.05$) compared to control values.

ineffective against behavior induced by NMDA at the highest dose of 5′-amino-5′-deoxyadenosine tested (1 nmol). In this study and our earlier studies (Keil and DeLander, 1992, 1994), we observed slight hind limb flaccidity at doses of 5′-amino-5′-deoxyadenosine greater than 1 nmol, although no gross motor deficits were observed. Doses of 5′-amino-5′-deoxyadenosine were, therefore, limited to 1 nmol or less to avoid possible motor effects. I.t. pretreatment (5 or 60 min) with an adenosine deaminase inhibitor, deoxycoformycin (200 nmol), failed to effect behavior induced by NMDA, AMPA, kainic acid or substance P (data not shown).

As shown in Fig. 2, theophylline (6.25–55.5 pmol) dose dependently reversed 5′-amino-5′-deoxyadenosine (1 nmol)-mediated inhibition of kainic acid- and substance P-induced biting and scratching. Importantly, theophylline reversed 5′-amino-5′-deoxyadenosine-mediated effects at doses which did not induce other behavioral effects if administered alone (see below).

Control, saline-injected (i.t.) mice displayed typical 'grooming' behavior commencing 1–2 min after placement in the observation chamber. Such behavior was casual in nature: grooming included the whole body, was interspersed by long periods of quiescence, and was not accompanied by vocalization. Grooming behavior in control mice which involved bites, licks or scratches toward

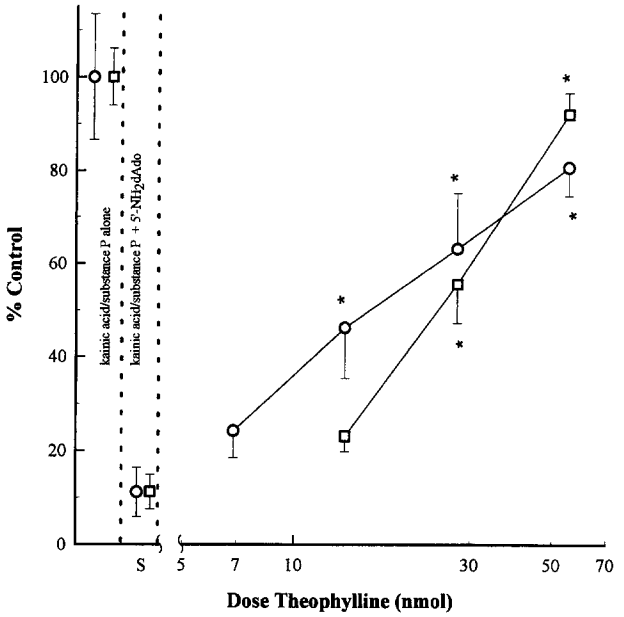

Fig. 2. Antagonism of 5′-amino5′-deoxyadenosine-mediated inhibition of kainic acid- or substance P-induced biting and scratching by the adenosine receptor antagonist, theophylline. Saline (S) or graded doses of theophylline were coadministered (i.t.) with kainic acid (○; 60 pmol) or substance P (▽; 6 pmol) and 5′-amino-5′-deoxyadenosine (1 nmol). Caudally directed biting and scratching behavior was quantitated over the next minute (mean ± S.E.M.; $n = 7–10$). * Significantly increased biting and scratching behavior when theophylline was coadministered compared to behavior seen following coadministration of kainic acid or substance P with 5′-amino-5′-deoxyadenosine ($P < 0.05$).

the hind quarters or tail, however, was quantitated for comparison to behavior induced by adenosine receptor antagonists. Increased biting and scratching behavior and occasional vocalizations was observed following i.t. adenosine receptor antagonist administration (Fig. 3). Theophylline-induced biting and scratching was not significantly different than control behavior following administration of 111 nmol, but significantly increased behavior

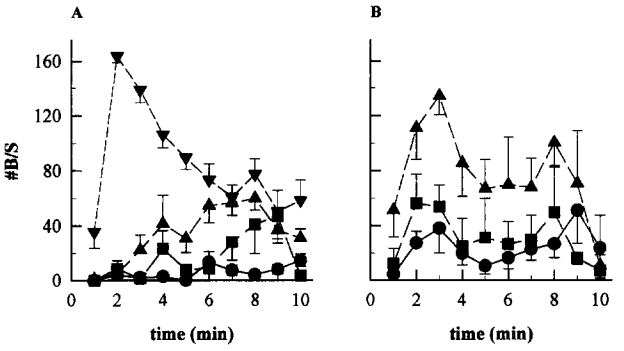

Fig. 3. Caudally directed biting and scratching behavior induced by the adenosine receptor antagonists, theophylline and 8-*p*SPtheophylline. Caudally directed biting and scratching (#B/S) behavior (mean ± S.E.M.; $n = 7–10$) was quantitated each min for 10 min following the administration (i.t.) of saline (●), theophylline [111 nmol (■) or 222 nmol (▲)] (A) or 8-*p*SPtheophylline [25 nmol (■), 50 nmol (▲) or 100 nmol (▼)] (B).

Table 1
Nociceptive behavior induced by adenosine receptor antagonists

I.t. administration [a]	ave # B/S [b]	I.t. administration	ave # B/S
N.S. [c]	24.6 (5.7)	Theophylline 222	66.4 (6.1)
Theophylline 111	30.7 (10.2)	Theophylline 222 + CPA [d]	50.7 (6.7) [f]
Theophylline 222	77.3 (8.3) [f]	Theophylline 222	69.7 (2.9)
		Theophylline 222 + NECA [e]	48.5 (5.3) [f]
D.W. [c]	5.7 (1.0)	8-*p*SPTheo 100	97.8 (3.6)
8-*p*SPTheo 25	16.9 (6.7) [f]	8-*p*SPTheo 100 + CPA	65.5 (9.1) [f]
8-*p*SPTheo 50	34.1 (3.4) [f]	8-*p*SPTheo 100	68.4 (11.2)
8-*p*SPTheo 100	85.5 (5.5) [f]	8-*p*SPTheo 100 + NECA	67.8 (12.9)

Dose dependency of adenosine receptor antagonists, theophylline and 8-*p*-sulphophenyl theophylline (8-*p*SPTheo), to induce caudally directed biting and scratching behavior (left columns), and inhibition of this behavior by pretreatment (10 min) with the adenosine receptor agonists, N^6-cyclopentyl adenosine (CPA) or 5′-(*N*-ethyl)-carboxamido adenosine (NECA) (right columns). [a] Doses listed are in nmol. [b] Caudally directed biting and scratching (# B/S) was observed for 10 min following i.t. administration of drugs and the means (± S.E.M.) behavior per min indicated. [c] N.S. = normal saline; d.w. = distilled water. [d] CPA dose = 100 pmol. [e] NECA dose = 25 pmol. [f] $P < 0.05$, paired Student's *t*-test.

was evident approximately 3 min following injection of 222 nmol theophylline (Fig. 3A). Behaviors gradually returned to baseline values by approximately 10 min. The more water-soluble antagonist, 8-*p*SPtheophylline, also induced caudally directed biting and scratching behavior (Fig. 3B) and vocalizations. Behavior induced by 100 nmol 8-*p*SPtheophylline was significantly increased during the first 5 min, returning to control values by approximately 10 min. Average behavior (min^{-1}) induced by theophylline and 8-*p*SPtheophylline over the first 10 min are summarized in Table 1.

Pretreatment (5 min) with the adenosine A_1 receptor-selective agonist, N^6-cyclopentyl adenosine (CPA; 100 pmol), or the non-selective adenosine receptor agonist, 5′-(*N*-ethyl)-carboxamido adenosine (NECA; 25 pmol), in doses which significantly inhibit excitatory amino acid- and SP-induced biting and scratching (DeLander and Wahl, 1988), had varying effects on methylxanthine-induced behavior. CPA significantly inhibited the biting and scratching behavior induced by theophylline and 8-*p*SPtheophylline and NECA significantly reduced theophylline-induced effects (Table 1). NECA, however, failed to significantly reduce average behaviors induced by 8-*p*SPtheophylline (Table 1), and appeared to either decrease or increase behavior at specific time points (data not shown).

Coadministration of excitatory amino acid receptor antagonists or the neurokinin receptor antagonist, spantide, also had varying effects on theophylline-induced biting and scratching behavior. Significant reductions were observed at selected time points following coadministration

Table 2
Sensitivity of theophylline-induced biting and scratching behavior to antagonists (antag) of excitatory amino acid [APV, MK-801, kynurenate, CNQX] or tachykinin [spantide (span)] receptors

	APV [a]	MK-801	Kynurenate	CNQX	Span	APV + span
Theophylline 222 [b]	54.9 (5.4) [c]	54.9 (5.4)	62.3 (2.2)	58.8 (3.4)	54.9 (5.4)	62.2 (9.1)
Theophylline 222 + antagonist	51.0 (5.3)	51.5 (4.0)	52.4 (5.9) [d]	64.7 (2.8)	39.4 (6.3) [d]	31.1 (3.6) [d]

[a] Doses of antagonists administered (pmol): APV – 600; MK-801 – 500; kynurenate – 250; CNQX – 5000; spantide (span) – 1000. [b] Theophylline dose = nmol. [c] Caudally directed biting and scratching was observed for 10 min following i.t. administration of drugs and the means (±S.E.M.) behavior per min indicated. [d] $P < 0.05$, paired Student's t-test.

of theophylline with APV, kynurenate, CNQX, and spantide (data not shown), but the overall number of bites and scratches over 10 min was significantly reduced only following kynurenate or spantide administration (Table 2). Coadministration of APV with spantide, however, appeared to enhance the efficacy of spantide, significantly reducing bites and scratches induced by theophylline at most time points (data not shown) and decreasing average biting and scratching over the 10 min observation period (Table 2).

In preliminary investigations, dose-dependent tactile allodynia was also observed following i.t. administration of graded doses of adenosine receptor antagonists (Fig. 4). Allodynia was not observed at any time point following administration of saline (i.t.) (data not shown). Light stroking of the hind quarters induced escape behavior, vocalization and touch-evoked biting and scratching following theophylline and 8-*p*SPtheophylline (i.t.) administration (Fig. 4). Allodynia was observed at early time points for all doses tested and persisted for more than 60 min at higher doses. Importantly, light stroking of rostral dermatotomes in theophylline- and 8-*p*SPtheophylline-treated mice failed to induce allodynic behavior, indicating a localized spinal effect.

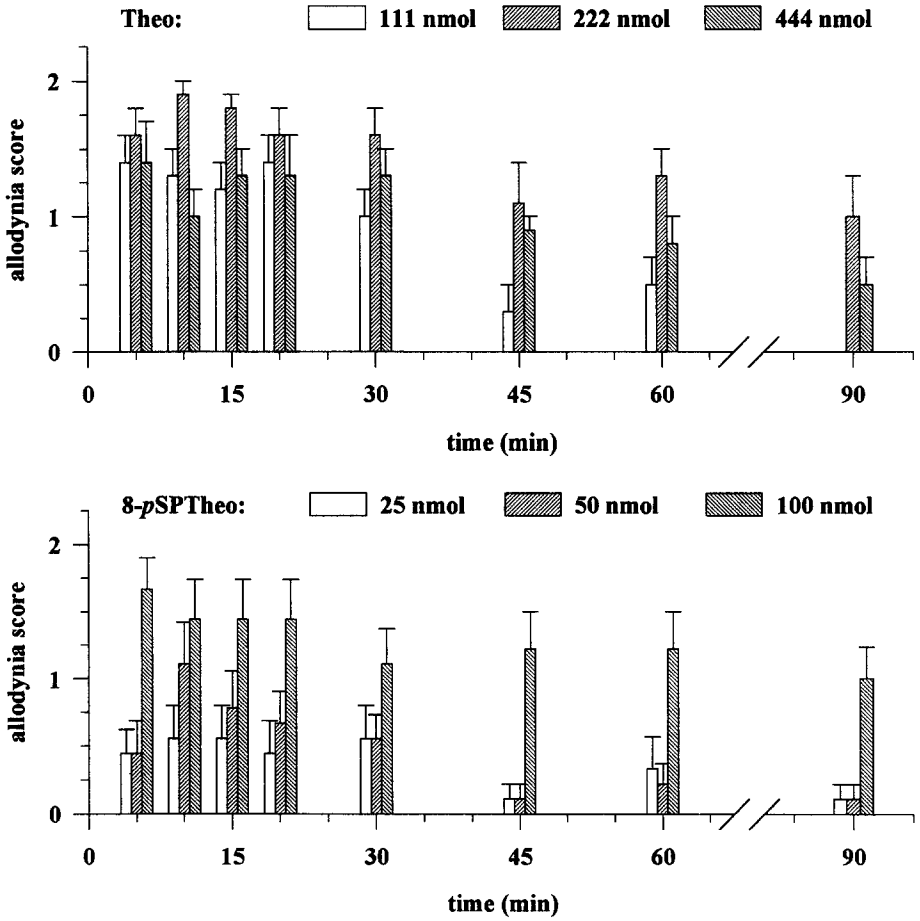

Fig. 4. Methylxanthine-induced tactile allodynia following i.t. administration. Mice ($n = 7–10$) were administered vehicles or graded doses of the adenosine receptor antagonists, theophylline of 8-*p*SPtheophylline (8-*p*SPTheo), and tactile allodynia assessed at selected time points. Behavior (±S.E.M.) was evaluated on the 3 point system described by Yaksh and Harty (1988). No effect was seen by vehicles in any animal tested at any time point and are not shown for clarity. (See Materials and methods for details.)

4. Discussion

Multiple studies have been conducted characterizing antinociception induced by adenosine or adenosine receptor agonists and the primary role of spinal sites in this pharmacologic action (Sawynok and Sweeney, 1989; Salter et al., 1993). Efforts to functionally define the existence and significance of a tonically active endogenous purinergic system in the modulation of sensory input are less complete. The present investigations were designed to provide additional support for a tonically active adenosine-mediated modulation of nociception and to begin to characterize how endogenous purinergic systems may interact with putative pain neurotransmitters.

Building upon observations in other model systems (Zhang et al., 1993), our laboratory has demonstrated that inhibition of adenosine kinase, in particular, appears to increase physiologic concentrations of endogenous adenosine and induce significant antinociception in thermal assays (Keil and DeLander, 1992, 1994). Similar observations were recently reported for antinociception in the formalin test (Poon and Sawynok, 1995). The present studies demonstrate, in an additional assay, that endogenous adenosine can be manipulated to induce antinociception. Behavior induced by i.t. administration of kainic acid or substance P was decreased by coadministration of 5′-amino-5′-deoxyadenosine, an inhibitor of adenosine kinase, in a dose-dependent, theophylline-reversible manner. Behavior induced by AMPA was partially inhibited. These studies parallel studies reported previously from our laboratory in which administration of exogenous adenosine analogues i.t. inhibited behaviors induced by putative pain neurotransmitters (DeLander and Wahl, 1988, 1989, 1991). These observations provide additional evidence supporting a hypothesis that endogenous adenosine modulates nociceptive input at spinal sites. Further, antinociception can be achieved by increasing endogenous adenosine concentrations through pharmacologic manipulations.

Inhibition of adenosine kinase did not inhibit behaviors induced by NMDA (i.t.) administration in the present study and this observation is in contrast to our earlier investigations with i.t. administration of exogenous adenosine analogues. Doses of 5′-amino-5′-deoxyadenosine utilized in the current investigations were limited to 1 nmol to avoid any potential complications in analysis due to motor effects. It seems unlikely, however, that this dose was inadequate. Doses of adenosine agonists administered i.t. required to inhibit biting and scratching behavior are quite small relative to the doses required for effects in thermal assays (DeLander and Hopkins, 1987). 1 nmol 5′-amino-5′-deoxyadenosine is adequate to cause effects in thermal assays (Keil and DeLander, 1992, 1994) and was effective against other putative pain neurotransmitters in this study. Alternatively, differences in the antinociceptive effect of exogenous adenosine analogue administration and increased endogenous concentrations of adenosine may reflect differing importance of adenosine in the regulation of nociceptive input mediated by different neurotransmitters. Regional distribution of adenosine kinase, relative to the distribution of adenosine receptors, at spinal sites has not been characterized. Manipulations to enhance endogenous adenosine may mimic a physiologic role for adenosine, while administration of exogenous adenosine may more accurately reflect a less selective neuronal inhibition resulting from dramatically increased release of adenosine during pathology.

Potentially differing roles for adenosine in physiology and pathology are also supported by the failure of deoxycoformycin, an inhibitor of adenosine deaminase, to inhibit biting and scratching behavior. The significance of adenosine deaminase may vary in different physiologic processes, as demonstrated in several reports (Phillis and Edstrom, 1976; Radulovacki et al., 1983; Ballarín et al., 1991). Most studies in which adenosine deaminase inhibition fails to have significant efficacy, however, reveal inhibition of adenosine deaminase does significantly enhance the actions of adenosine administered exogenously (cf. Davies et al., 1982; Zhang et al., 1993; Keil and DeLander, 1994). Again, these observations may suggest the role of adenosine is dependent on the amount of adenosine present.

Inhibition of adenosine receptors using adenosine receptor antagonists is proposed to facilitate nociceptive behavior (Paalzow and Paalzow, 1973; Jurna, 1984; Sawynok et al., 1986; Paalzow, 1994). Our observations also revealed biting and scratching behavior following i.t. administration of theophylline or 8-*p*SPtheophylline and supports the existence of 'purinergic tone' at spinal sites. Pretreatment with CPA, and to a lesser extent NECA, inhibited antagonist-induced biting and scratching, again suggesting an adenosine receptor-mediated mechanism. The lesser efficacy of NECA is difficult to reconcile, but may reflect contrasting actions induced by NECA interactions with both A_1 and A_2 receptors.

Methylxanthines are reported to have a variety of actions unrelated to their antagonism of adenosine receptors, including inhibition of phosphodiesterase and 5′-nucleotidase, alterations in intracellular Ca^{2+} concentrations and modulation of GABA or noradrenergic neurotransmission (reviewed, Nehlig et al., 1992; reviewed, Sawynok and Yaksh, 1993). The potential for alternative mechanisms of action is raised by reports in which a membrane permeable cAMP analog, 8-Br cAMP, or inhibition of phosphodiesterase by 3-isobutyl-1-methylxanthine enhance depolarization of dorsal horn neurons (Cerne et al., 1992). Evaluation of theophylline and its comparison to 3-isobutyl-1-methylxanthine determined previously by our laboratory, however, has not supported a mechanism based on inhibition of phosphodiesterase (DeLander and Hopkins, 1986). Additionally in the present study, administration of 8-*p*SPtheophylline induced behaviors similar to theophylline. 8-*p*SPtheophylline is largely limited to extracellular sites

of action due to its hydrophilic nature, again suggesting blockade of membrane-bound adenosine receptors as the primary mechanism of action.

Disruption of purinergic tone by adenosine receptor antagonists may disinhibit excitatory pathways mediating nociception. Both pre- and post-synaptic effects for spinal adenosine have been proposed (Li and Perl, 1994). Results from the present investigations were generally consistent with disinhibition of excitatory pathways following methylxanthine administration. Coadministration of excitatory amino acid receptor antagonists with adenosine receptor antagonists decreased biting and scratching behavior at selected time points, but inhibition of overall behavior was demonstrated only for kynurenate, a non-selective excitatory amino acid antagonist and spantide, a tachykinin receptor antagonist. Other investigators have directly observed no effect (Vasko and Ono, 1990) or decreased (Santicioli et al., 1993) substance P release and decreased calcitonin gene-related peptide release (Santicioli et al., 1992, 1993) from primary afferents following treatment with adenosine analogues.

Discussions by Urban et al. (1994) of interactions between excitatory amino acids and tachykinins in neurotransmission may help to explain certain results in the present investigations. The greatest inhibition of methylxanthine-induced behavior observed in our investigations was achieved when antagonists of an excitatory amino acid receptor and a tachykinin receptor were coadministered. Inhibition of a single receptor class may be inadequate to induce consistent behavioral effects. Recent papers by Poon and Sawynok (1995) and Reeve and Dickenson (1995a,b) also suggest stimulus intensity in specific assays is an important determinant in revealing the efficacy of endogenous or exogenous adenosine in sensory processing.

The existence of a tonic role for endogenous adenosine raises the possibility that its role may extend beyond modulation of nociceptive input. Removal of an inhibitory purinergic tone may result not only in facilitated nociceptive neurotransmission (i.e. hyperalgesia), but also the miscoding of innocuous stimuli (i.e. allodynia). In preliminary experiments, we observed allodynia following i.t. administration of adenosine receptor antagonists. Interestingly, spinal (Sosnowski et al., 1989; Minami et al., 1992) and systemic (Belfrage et al., 1995; Segerdahl et al., 1995; Sollevi et al., 1995) adenosine administration has been efficacious in treating allodynia in both animals and humans.

The present investigations provide additional support for the existence of an endogenous purinergic system tonically modulating nociceptive input. Inhibition of purinergic tone may disinhibit excitatory neuronal pathways, and our results reinforce the hypothesis that nociceptive behavior is likely a composite resulting from concurrent activation of several nociceptive pathways. Finally, in preliminary investigations, observations concur with clinical reports suggesting perturbation of purinergic tone may contribute to allodynia. Restoration of spinal adenosine neuromodulation either by administering adenosine analogues exogenously or by enhancing endogenous adenosine concentrations may represent a novel therapeutic approach to the treatment of pain syndromes.

Acknowledgements

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