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Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats

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Abstract

Inhibitors of adenosine kinase (AK) enhance extracellular concentrations of the inhibitory neuromodulator adenosine (ADO) at sites of tissue hyperexcitability and produce antinociceptive effects in animal models of pain and inflammation. The present study compared the ability of several novel and selective AK inhibitors and ADO receptor-selective agonists to attenuate carrageenan-induced thermal hyperalgesia and to impair motor performance as measured by effects on exploratory motor activity and rotorod performance. The prototypical nucleoside AK inhibitor, 5′deoxy-5-iodotubercidin (5′d-5IT), dose-dependently blocked thermal hyperalgesia (ED_{50} = 0.2 μmol/kg ip) and was 4- and 75-fold less potent in reducing exploratory motor activity and rotorod performance, respectively. The antihyperalgesic effects of 5′d-5IT were fully blocked by the A_1 antagonist, cyclopentyltheophylline (CPT) and the A_{2A} antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX). Novel nucleoside and non-nucleoside AK inhibitors (A-134974, A-286501 and ABT-702) also potently (ED_{50} = 0.7–2 μmol/kg ip) blocked carrageenan-induced thermal hyperalgesia and were significantly less potent than 5′d-5IT in impairing motor performance. The systemic administration of N^6-cyclopentyladenosine (CPA), an A_1 receptor-selective agonist, CGS 21680, an A_{2A} receptor-selective agonist, and N^6-ethylcarboxamidoadenosine (NECA), a nonselective ADO receptor agonist potently reduced (ED_{50} = 0.3–1.0 μmol/kg ip) thermal hyperalgesia. Unlike the AK inhibitors, however, these ADO receptor agonists produced significant antinociception only at doses that also decreased motor performance. These data demonstrate that AK inhibitors produce specific antihyperalgesic effects via an interaction with ADO A_1 and A_{2A} receptors at doses that lack detectable effects on exploratory motor activity and rotorod performance and offer an improved separation between antinociceptive and motor impairing effects as compared to ADO receptor agonists.

Keywords: Adenosine; Adenosine kinase; Analgesia; Hyperalgesia; Nociception

1. Introduction

The systemic administration of adenosine (ADO) and ADO receptor agonists provide clinically effective analgesia by reducing anesthesia requirements perioperatively, and opioid use following surgery (Segerdahl and Sollevi, 1998; Sollevi, 1997; Segerdahl et al., 1997). It has also been recently reported that systemic ADO administration can reduce opioid-resistant neuropathic pain in humans (Gyllenhammar and Norfors, 2001). Consistent with these clinical observations, ADO and ADO receptor agonists have analgesic actions in a wide range of animal studies including models of acute thermal somatic pain (Holmgren et al., 1986; Keil and Delander, 1992; Sawynok, 1997; Kowaluk et al., 1999), chemically induced persistent pain (Malmberg and Yaksh, 1993), inflammatory pain (Poon and Sawynok, 1998) and models of nerve injury-induced pain (Lee and Yaksh, 1996; Lavand'Homme and Eisenach, 1999). ADO receptors (Choca et al., 1987), ADO immunoreactivity (Braas et al., 1986), ADO deaminase (ADA) (Gieger and Nagy, 1986) and ADO transporters (Gieger and Nagy, 1985) are found in the spinal cord and appear to be a primary site of action of ADO mediated analgesia (Sawynok et al., 1986; Reeve and Dick-

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enson, 1995; Keil and DeLander, 1992; McGaraughty et al., 2001a). Given the wide distribution of ADO receptors in the neuraxis, the clinical utility of direct acting ADO receptor agonists as analgesics is limited by receptor mediated central nervous system (motor impairment) and peripheral (hemodynamic) side effects (Williams and Jarvis, 2000).

Since ADO concentrations are tightly regulated at cellular sites where it is released (Moser et al., 1989), inhibition of the primary metabolic enzyme for ADO, adenosine kinase (AK; ATP: adenosine 5′-phosphotransferase, EC 2.7.1.20), represents an alternative strategy to facilitate the beneficial actions of ADO. AK inhibitors have been demonstrated to increase extracellular ADO concentrations in vitro (White, 1996; Golembiowska et al., 1996) and to selectively increase ADO concentrations in vivo in traumatized neural (Britton et al., 1999) and peripheral (Liu et al., 2000) tissues. AK inhibitors have also been shown to be more effective than adenosine deaminase inhibitors in elevating extracellular ADO concentrations (Pak et al., 1994; Golembiowska et al., 1996) and in reducing nociception (Keil and Delander, 1992; Poon and Sawynok, 1995).

The ability of AK inhibitors to selectively enhance ADO availability in a site and event specific fashion in response to neuronal excitability (Britton et al., 1999) suggests that these agents may provide an increased therapeutic window as compared to direct-acting ADO receptor agonists (Engler, 1987; Mullane and Young, 1993; Kowaluk and Jarvis, 2000). This hypothesis is supported by recent data indicating that systemically administered AK inhibitors can reduce nociception, seizure susceptibility and anesthetic requirement in animals at doses that do not alter cardiovascular function (Wang et al., 1997; Wiesner et al., 1999; Kowaluk et al., 2000).

The present report compares the antinociceptive and motor impairing effects of several novel AK inhibitors (Fig. 1) with the actions of cyclopentyladenosine (CPA), an ADO A_1 receptor selective agonist, CGS 21680, an A_{2A} receptor selective agonist, and N^6-ethylcarboxamidoadenosine (NECA), a nonselective ADO receptor agonist. Recent data from our laboratory have shown that AK inhibitors are more potent in attenuating inflammation-induced thermal hyperalgesia as compared to their potency in reducing acute and neuropathic nociception (Kowaluk et al., 2000; Jarvis et al., 2002). The present data demonstrate that direct acting ADO agonists reduce thermal hyperalgesia, but at doses that also alter motor performance. In contrast, AK inhibitors reduce thermal hyperalgesia at doses that are generally devoid of motor impairment.

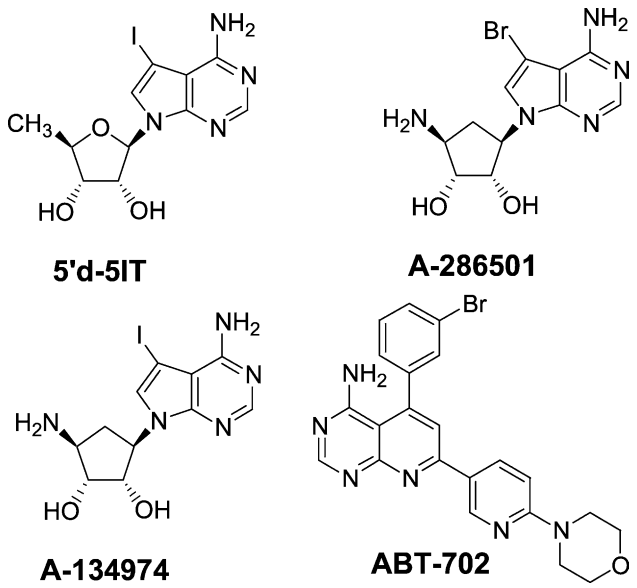

Fig. 1. Structures of the AK inhibitors.

2. Methods

2.1. Materials

[U-^{14}C]Adenosine (542 mCi/mmol) and [2-^{3}H]adenosine (26 Ci/mmol) were purchased from Amersham International (Amersham, Buckinghamshire, United Kingdom). Bovine serum albumin, ATP, ADO and other chemical reagents were purchased from Sigma (St. Louis, MO). A-286501 and A-134974 were synthesized as described by Bhagwat and Cowart (2000). ABT-702 was synthesized as previously described (Lee et al., 2001) and was dissolved in a vehicle consisting of 10% dimethylsulfoxide/34% hydroxypropyl-β-cyclodextrin in water. 5′-Deoxy-5-iodotubercidin (5′d-5IT) (Sigma), A-134974 and A-286501 were dissolved in water. ADO agonists and antagonists were obtained from Sigma and dissolved in 9% saline. Doses are expressed in μmol/kg of freebase and compounds were administered in a final volume of 1–3 ml/kg ip.

2.2. Subjects

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 200–300 g were utilized for all experiments. These animals were group housed (four per cage) in AAALAC approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 0700 and 2000 h. Food and water was available ad libitum except during testing. Animals were used only once in each experiment and individual groups ($n=6$ per group) were treated with either test compound or vehicle. All animal handling and experimental protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

2.3. In vitro assays

AK enzyme inhibition and ADO phosphorylation in intact cells were assayed as described by Jarvis et al. (2000). Briefly, AK inhibition was measured at 23 °C in a 100 μl reaction mixture in triplicate containing 64 mM Tris–HCl (pH 7.5), 0.2 mM $MgCl_2$, 1 mM ATP, 0.2 μM [U-^{14}C]adenosine or [2-^{3}H]adenosine (Amersham International) and

appropriate volumes of rat brain cytosol as a source of AK. After incubation for 15 min, the reaction was terminated by aliquoting 40 μl of the reaction mixture onto DE-81 anion exchange filter disks. The filter disks were air-dried, washed in 2 mM ammonium formate and dried again. Bound radioactivity was determined by standard scintillation spectrometry. Assays for ADO phosphorylation in intact cells were conducted using confluent IMR-32 human neuroblastoma cells (ATCC, Gaithersburg, MD). Appropriate concentrations of test compounds (10^{-11} to 10^{-4} M) were added to each cell culture well and incubated in 400 μl warm Gey's Balanced Salt Solution for 10 min. The reaction run in triplicate was initiated by the addition of 50 μl 2 μM [U-^{14}C]adenosine. After a 20-min incubation, the assay buffer was rapidly aspirated and the cells were quickly frozen by the addition of excess liquid nitrogen. A 50-μl aliquot of the thawed supernatant was placed onto DE-81 filter disks and processed as described above. Radioligand binding assay methodology for the A_1, A_{2A} and A_3 receptors was carried out as described by Jarvis (1998). The ability of AK inhibitors to inhibit [^{3}H]nitrobenzylthioinosine (NBTI) binding to the ADO transporter and to inhibit adenosine deaminase activity was also examined using previously described methodology (Parkinson and Geiger, 1996).

2.4. Thermal nociception and carrageenan-induced thermal hyperalgesia

Carrageenan-induced hyperalgesia was induced by injecting 100 μl of a 1% solution of λ-carrageenan (Sigma) in physiological saline into the plantar surface of the right hindpaw of the rat as previously described (Kowaluk et al., 2000). Paw withdrawal latencies of both injured and uninjured paws to thermal stimulation was determined 2 h later using a commercially available paw thermal stimulator (UARDG, Department of Anesthesiology, University of California, San Diego, La Jolla, CA), modeled after that described by Hargreaves et al. (1988). Test compounds or vehicle were administered intraperitoneally 30 min before carrageenan to individual treatment groups ($n = 6$ per group). In antagonist experiments, ADO receptor antagonists were administered intraperitoneally 15 min after 5′d-5IT administration. Paw withdrawal latencies were calculated as the mean of the two shortest latencies.

2.5. Locomotor activity and rotorod performance

Exploratory locomotor activity was measured in an open field using photobeam activity monitors (AccuScan Instruments, Columbus, OH). Rats were treated with test compounds or vehicle intraperitoneally and placed in activity chambers ($42 \times 42 \times 30$ cm) 30 min later. Photobeam breaks were recorded for 30 min and data were collapsed into 10-min intervals for statistical analysis. Rotorod performance was measured using an accelerating rotorod apparatus (AccuScan Instruments). Rats were allowed a 30-min acclimation period in the testing room and then placed on a 9-cm diameter rod that increased in speed from 0 to 20 rpm over a 60-s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given three training sessions before drug treatment. Following the training sessions, rats were randomly assigned to treatment groups ($n = 6$ per group) and injected with either test compounds or vehicle. Latencies to fall from the rotorod were determined 60 min following test compound or vehicle treatment.

2.6. Statistics

Individual dose response data was analyzed using one-way analysis of variance (GB-Stat, Dynamic Microsystems, Silver Spring, MD) as previously described (Kowaluk et al., 1999). Where appropriate, Fisher's protected least significant difference (FPLSD) was used for post-hoc analysis of all possible comparisons of means. The level of significance was set at $P < .05$. ED_{50} values were estimated using least squares linear regression. Data are presented as mean ± S.E.M.

3. Results

All four AK inhibitors had high affinity to block AK activity in both the enzyme assay and in an intact cell assay (Table 1). These compounds were also significantly more potent to inhibit AK activity as compared to their ability to inhibit ADA activity or radioligand binding to ADO receptor subtypes or the ADO transporter (Table 1). The structurally novel AK inhibitors, ABT-702, A-134974 and A-286501, were also found to be significantly less active (1500- to

Table 1
Summary pharmacology of AK inhibitors and ADO agonists

Compound	AK	Intact cell	A_1	A_{2A}	A_3	NBTI	ADA
IC_{50} (nM)							
5′d-5IT	0.9 ± 0.1	68.1 ± 7.5	>10,000	>10,000	>10,000	>10,000	>10,000
A-134974[a]	0.06 ± 0.07	45 ± 9	>10,000	>10,000	>10,000	>10,000	>10,000
A-286501[a]	0.5 ± 0.1	12 ± 1	>10,000	>10,000	>10,000	>10,000	>10,000
ABT-702[a]	1.7 ± 0.5	51 ± 8	>10,000	2110 ± 1000	>10,000	2220 ± 370	>10,000

Data represent mean ± S.E.M. from at least three individual experiments. Compounds were evaluated for their ability to inhibit AK from rat brain cytosol and to inhibit ADO phosphorylation in intact IMR-32 cells. Compounds were also evaluated for their ability to inhibit ADO deaminase (ADA) and to compete for radioligand binding at ADO receptors and the ADO transporter site (NBTI).

[a] Data from Jarvis et al. (2000, 2002) and McGaraughty et al. (2001a).

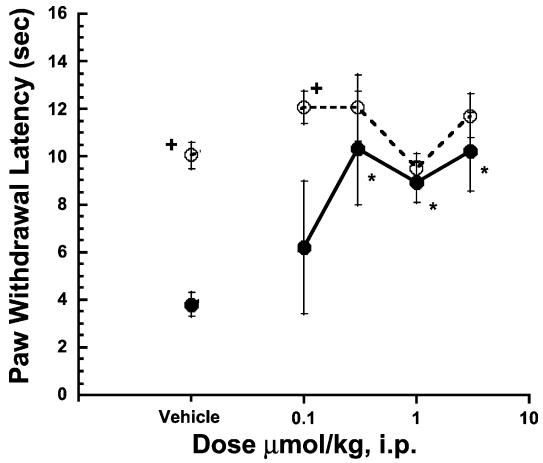

Fig. 2. Effects of 5′d-5IT to attenuate carrageenan-induced thermal hyperalgesia in the rat. Filled circles represent mean (±S.E.M., $n=6$) paw withdrawal latencies of the carrageenan-injected (injured) paw. Open circles represent mean (±S.E.M., $n=6$) responses paw withdrawal latencies of the contralateral (uninjured) paw. 5′d-5IT (0.1, 0.3, 1 and 3 μmol/kg ip) was administered 30 min before carrageenan administration. * $P<.05$ versus vehicle. $^{+}P<.05$ versus contralateral normal paw.

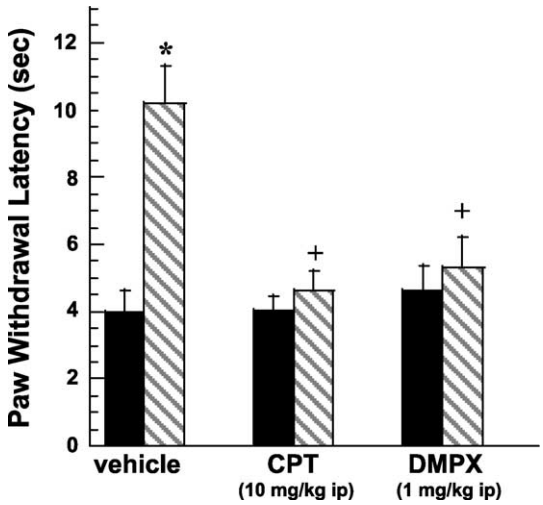

Fig. 3. Effects of CPT (10 mg/kg ip) and DMPX (1 mg/kg ip) to block 5′d-5IT (1 μmol/kg ip)-induced antihyperalgesia. Black bars represent mean (±S.E.M., $n=6$) paw withdrawal latencies of injured (carrageenan-injected) paws from rats receiving drug vehicle. Hatched bars represent mean (±S.E.M., $n=6$) paw withdrawal latencies of 5′d-5IT in the absence (vehicle) or presence of ADO receptor antagonists. * $P<.05$ versus vehicle. $^{+}P<.05$ versus 5′d-5IT.

>10,000-fold) across a range of other neurotransmitter receptors, enzymes and ion channels (Jarvis et al., 2000, 2002 and data not shown). In contrast, the ADO receptor agonists did not inhibit AK at concentrations up to 10 μM (data not shown).

Intraplanar administration of carrageenan produced a significant increase in sensitivity to thermal stimulation as indicated by a decrease in the mean paw withdrawal latency from 10.1±0.5 to 3.8±0.5 s ($P<.05$, Fig. 2). Systemic administration of the prototypical AK inhibitor, 5′d-5IT dose-dependently blocked carrageenan-induced thermal hyperalgesia ($ED_{50}=0.2$ μmol/kg ip) in the rat (Fig. 2 and Table 2). Doses of 5′d-5IT greater than or equal to 0.3 μmol/kg ip fully blocked carrageenan-induced hyperalgesia and these antinociceptive effects were specific to the injured paw as no significant drug effects ($P>.05$) were observed for the uninjured contralateral paw. Systemic administration of the A_1 receptor-selective antagonist, cyclopentyltheophylline (CPT, 10 mg/kg ip) fully blocked the antihyperalgesic actions of 5′d-5IT (Fig. 3). The antinociceptive effects of 5′d-5IT were also fully blocked by the systemic administration of an A_{2A} receptor-selective dose (Seale et al., 1988) of 3,7-dimethyl-1-propargylxanthine (DMPX, 1 mg/kg ip) (Fig. 3).

Systemic administration of 5′d-5IT also dose-dependently ($P<.05$) reduced exploratory motor activity and rotorod performance in the rat (Fig. 4). 5′d-5IT was approximately 20-fold more potent in reducing exploratory motor

Table 2
Activity of AK inhibitors and ADO agonists to attenuate hyperalgesia and impair motor performance

	Assay ED_{50} (μmol/kg ip)			Ratio of effect	
	Carrageenan hyperalgesia	Locomotor activity	Rotorod activity	Locomotor/ Hyperalgesia	Rotorod/ Hyperalgesia
AK inhibitors					
5′d-5IT	0.2	0.7	15	3.5	75
A-134974	1.0	16	>30	16	>30
ABT-702	0.7	7	>100	10	>100
A-286501	2	20	70	10	35
ADO agonists					
A_1-CPA	0.7	3	30	4.3	43
A_{2A}-CGS 21680	1	2	>30	2	>30
A_1/A_2-NECA	0.3	0.5	7	1.7	23

Data represent ED_{50} values for AK inhibitors and ADO receptor agonists to reduce carrageenan-induced hyperalgesia or motor performance (locomotor and rotorod activity). The ratio of effect represents the degree of separation between the potency of these compounds in reducing motor performance as compared to their antihyperalgesic effects.

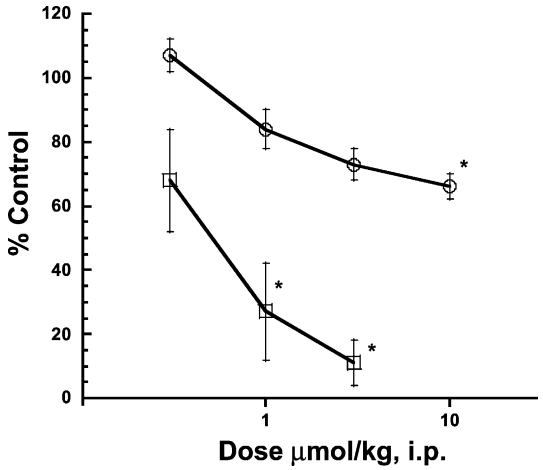

Fig. 4. Effects of 5′d-5IT to reduce locomotor activity (open squares, $n=6$) and rotorod performance (open circles, $n=6$). Percent control represents drug effects relative to vehicle treated rats. Locomotor activity (total number of photobeam breaks) in vehicle-treated rats averaged 4436±768 ($n=6$). The mean fall latencies for vehicle treated ($n=6$) rats in the rotorod assay were 48.6±2.4 s. 5′d-5IT (0.3, 1, 3 or 10 μmol/kg ip) was administered 30 min before testing. * $P<.05$ versus vehicle responses.

activity ($ED_{50}=0.7$ μmol/kg ip) as compared to its effects on rotorod performance ($ED_{50}=15$ μmol/kg ip). At a dose of 3 μmol/kg ip, 5′d-5IT produced almost complete inhibition of exploratory motor activity, however, rats were awake, responsive to stimuli and retained the righting reflex consistent with their ability to perform the rotorod task. This dose of 5′d-5IT did not produce a significant decrease in rotorod performance, however, a significant ($P<.05$) 30% reduction in rotorod performance was produced by 10 μmol/kg ip 5′d-5IT (Fig. 4). Taken together, these data demonstrate that systemically administered 5′d-5IT does impair motor performance as measured by the locomotor and rotorod assays, but 5′d-5IT was, respectively, 4- and 75-fold more potent in reducing carrageenan-induced thermal hyperalgesia (Table 2). Thus, at a dose of 0.3 μmol/kg ip that fully blocked thermal hyperalgesia, no significant alteration in motor performance (Fig. 4) or responses on the contralateral (uninjured) paw (Fig. 2) were observed.

Additional experiments were conducted to compare the antihyperalgesic and motor impairing effects of several novel nucleoside and non-nucleoside AK inhibitors with the actions of several ADO receptor agonists following intraperitoneal administration. Similar to the analgesic actions of 5′d-5IT, the AK inhibitors, A-286501, A-134974 and ABT-702 dose-dependently blocked carrageenan-induced thermal hyperalgesia (Fig. 5 and Table 2). These AK inhibitors also produced reductions in exploratory motor activity and in rotorod performance (Fig. 5). However, these latter actions occurred at doses that were 10- to >100-fold higher than were required to attenuate thermal hyperalgesia (Table 2). At intraperitoneal doses that completely blocked thermal hyperalgesia, A-134974 and ABT-702 did not significantly alter rotorod performance and produced an approximately 30% decrease in locomotor activity. Similarly, A-286501 did not alter locomotor activity and produced only a 20% decrease in rotorod performance at doses that completely blocked thermal hyperalgesia (Fig. 5). These novel AK inhibitors also showed a greater separation between their antihyperalgesic and motor impairing effects as compared to 5′d-5IT (Table 2).

CPA, which has high selectivity for A_1 receptors, and CGS 21680, which has high selectivity for A_{2A} receptors (Jarvis, 1998), both dose-dependently ($P<.05$) reduced carrageenan-induced thermal hyperalgesia (Fig. 6). Additionally, the nonselective ADO agonist, NECA was two- to three-fold more potent in reducing thermal hyperalgesia as compared to CPA and CGS 21680 (Fig. 6 and Table 2). Like

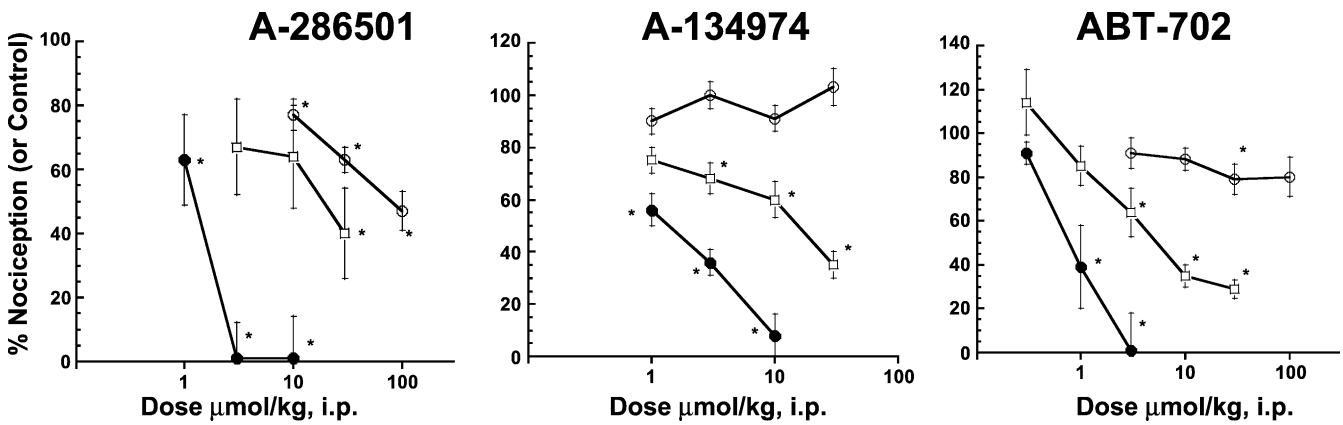

Fig. 5. Effects of AK inhibitors to attenuate thermal hyperalgesia (filled circles, $n=6$ per dose group), locomotor activity (open squares, $n=6$ per dose group) and rotorod performance (open circles, $n=6$ per dose group). One hundred percent nociception was defined at the mean paw withdrawal latency of the injured (carrageenan-injected) paw following intraperitoneal vehicle administration and ranged from 3 to 4 s across individual experiments. Locomotor activity (total number of photobeam breaks) in vehicle-treated rats ranged from 4000 to 5300 and the mean fall latencies for vehicle treated rats in the rotorod assay ranged from 42 to 50 s across individual experiments. Doses of A-286501 were 1, 3, 10, 30 and 100 μmol/kg ip. Doses of A-134974 were 1, 3, 10 and 30 μmol/kg ip. Doses of ABT-702 were 0.3, 1, 3, 10, 30 and 100 μmol/kg ip. * $P<.05$ versus vehicle responses, $n=6$ per dose group.

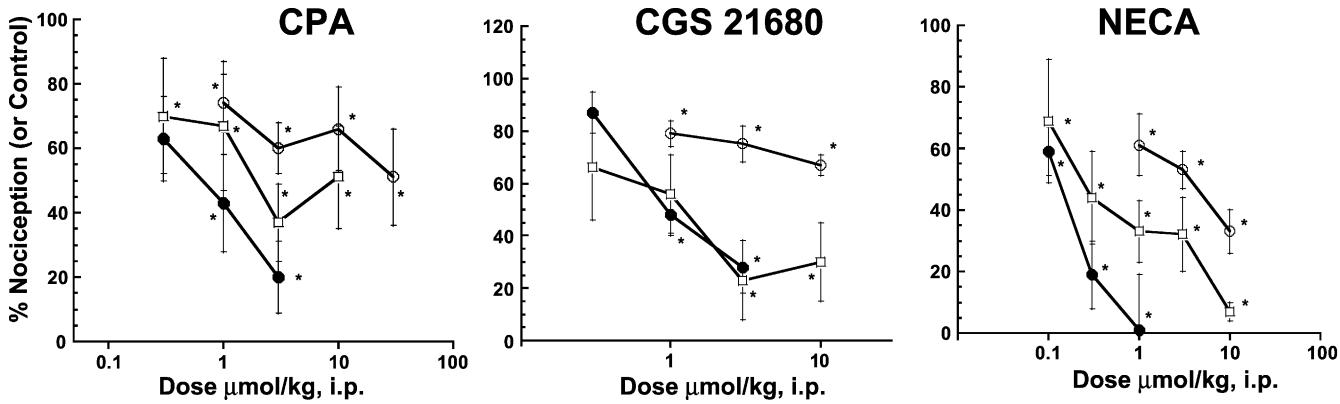

Fig. 6. Effects of ADO receptor agonists to attenuate thermal hyperalgesia (filled circles, $n=6$ per dose group), locomotor activity (open squares, $n=6$ per dose group) and rotorod performance (open circles, $n=6$ per dose group). One hundred percent nociception was defined as the mean paw withdrawal latency of the injured (carrageenan-injected) paw following intraperitoneal vehicle administration and ranged from 3 to 4 s across individual experiments. Locomotor activity (total number of photobeam breaks) in vehicle-treated rats ranged from 4000 to 5300 and the mean fall latencies for vehicle-treated rats in the rotorod assay ranged from 42 to 50 s across individual experiments. Doses of CPA were 0.3, 1, 3, 10 and 30 μmol/kg ip. Doses of CGS 21680 were 0.3, 1, 3 and 10 μmol/kg ip. Doses of NECA were 0.1, 0.3, 1, 3 and 10 μmol/kg ip. * $P<.05$ versus vehicle responses, $n=6$ per dose group.

5′d-5IT, these ADO receptor agonists did not alter responses of the contralateral (uninjured) paw in the thermal hyperalgesia assay indicating that the observed antinociceptive effects were specific to the injured paw (data not shown). All three ADO agonists also dose-dependently reduced exploratory motor activity and rotorod performance (Fig. 6). Unlike the AK inhibitors, the minimally effective antihyperalgesic doses of CPA, CGS 21680 and NECA occurred at doses that also produced significant motor impairment (Fig. 6). While these agonists were more potent in reducing thermal hyperalgesia as compared to their ability to disrupt motor performance, the degree of separation between their antihyperalgesic and motor effects was less than that for the AK inhibitors (Table 2).

4. Discussion

The present data demonstrate that both AK inhibitors and ADO agonists potently reduce thermal hyperalgesia following systemic administration. These data are consistent with the previously documented actions of ADO and ADO receptor agonists to reduce nociception in experimental animal pain models and in the clinical setting (Segerdahl and Sollevi, 1998; Sawynok, 1999). However, the present data highlight a major problem with the approach of using direct acting ADO receptor agonists as analgesic agents. While there is an abundant literature demonstrating that activation of ADO receptors can produce antinociceptive effects (see reviews by Sawynok, 1999; Kowaluk and Jarvis, 2000), the antinociceptive effects of systemically administered ADO agonists are typically accompanied by other CNS-mediated side effects, most notably impairments in motor performance (Herrick-Davis et al., 1999; Lee and Yaksh, 1996). This situation has led to the exploration of ADO administered intrathecally in both animals (Lee and Yaksh, 1996) and humans (Segerdahl and Sollevi, 1998) in an attempt to minimize CNS sedation.

As an alternative, inhibition of AK represents one approach to enhance the beneficial actions of ADO while reducing the nonspecific effects of general ADO receptor activation. This approach is predicated on the idea that inhibition of AK can preferentially enhance extracellular ADO concentrations in traumatized (or hyperexcited) tissues and cellular sites that are undergoing increased ADO turnover (Engler, 1987; Arch and Newsholme, 1978). This hypothesis is supported by data showing that AK inhibitors can effectively enhance ADO concentrations in injured CNS (Britton et al., 1999) and peripheral (Liu et al., 2000) tissues. Further, these actions are specific to sites of injury since AK inhibition selectively increases ADO concentrations in injured but not uninjured neuronal tissue (Britton et al., 1999). The present data show that these neurochemical effects may be physiologically relevant since AK inhibitors, but not ADO receptor agonists, can produce significant reductions in inflammatory thermal hyperalgesia at doses that do not have profound effects on motor performance. AK inhibitors have also been shown to produce robust antinociception and anticonvulsant activity in rodents in the absence of detectable alterations in hemodynamic function (Wiesner et al., 1999; Kowaluk et al., 2000; Jarvis et al., 2001).

Recently, a number of structurally novel nucleoside and non-nucleoside AK inhibitors have been developed that offer improved stability, cellular penetration, oral bioavailability and pharmacological specificity as compared to earlier compounds based on tubercidin (Davies et al., 1984; McGaraughty et al., 2001b). Evaluation of these compounds for their antinociceptive activity has shown that AK inhibitors exhibit a broad range of antinociceptive effects in animal models of acute, inflammatory and neuropathic pain, but are particularly potent to reduce inflam-

matory thermal hyperalgesia (Kowaluk et al., 2000; Jarvis et al., 2000, 2002; McGaraughty et al., 2001a; Zhu et al., 2001). The present data show that, unlike the ADO receptor agonists, the antihyperalgesic effects produced by AK inhibitors occur at doses that do not produce marked disruption of motor performance. The antihyperalgesic effects of the AK inhibitors also do not appear to be secondary to their anti-inflammatory effects (Kowaluk et al., 2000; Jarvis et al., 2002; Boyle et al., 2000) since the antinociceptive effects are evident at significantly lower doses than are required to reduce inflammation (Kowaluk et al., 2000; Jarvis et al., 2002). These novel AK inhibitors also showed a generally larger separation between antinociceptive and motor impairing effects as compared to the prototypical AK inhibitor, 5′d-5IT.

The antihyperalgesic effects of 5′d-5IT were fully blocked by both A_1 and A_{2A} receptor-selective antagonists. This antagonist pharmacology agrees with similar data obtained for blocking the antihyperalgesic actions of ABT-702, a non-nucleoside AK inhibitor (Kowaluk et al., 2000) and is consistent with fact that both CPA and CGS 21680 dose-dependently reduced thermal hyperalgesia in the present study. While spinal A_1 receptor activation appears to be the predominate ADO-mediated antinociceptive mechanism in acute and neuropathic pain (Sawynok, 1999; Lee and Yaksh, 1996; Jarvis et al., 2000; Kowaluk et al., 1999, 2000), the activation of multiple ADO receptor subtypes has been implicated in the homeostatic response to inflammation (Firestein, 1996). Both A_1 and A_{2A} receptors are localized in the spinal dorsal horn (Choca et al., 1987) and activation of A_{2A} receptors has been implicated in the antinociceptive effects of spinally administered ADO (Sawynok, 1999). The increased potency of AK inhibitors to reduce inflammatory hyperalgesia, as compared to other nociceptive states, suggests that activation of both A_1 and A_{2A} receptors may provide convergent mechanisms to alleviate inflammatory pain. The apparent two- to three-fold increased potency of the nonselective ADO agonist, NECA, to reduce thermal nociception as compared to the A_1 selective agonist CPA or the A_{2A} selective agonist CGS 21680 also supports this hypothesis. The ability of both AK inhibitors and ADO receptor agonists to reduce thermal hyperalgesia provides additional support for a role of ADO receptor activation in modulating central and peripheral nervous system sensitization following inflammation. These centrally mediated effects of ADO receptor activiation contrast other data indicating that the peripheral activation of A_{2A} receptors can produce pronociceptive responses (Taiwo and Levine, 1990; Doak and Sawynok, 1995) suggesting that central activation of ADO receptor subtypes may override the effects of ADO receptor activation on peripheral sensory afferent neurons.

While AK inhibitors show greater separation in their ability to reduce thermal hyperalgesa and motor performance as compared to ADO receptor agonists, both classes of compounds were less potent to reduce rotorod performance as compared to their ability to reduce locomotor activity. Both classes of compounds disrupt motor performance at sufficiently high doses, however, neither AK inhibitors nor ADO receptor agonists altered responses of the contralateral (uninjured) paw in the thermal hyperalgesia assay. Drug effects on the contralateral paw in the thermal hyperalgesia assay may reflect an overt analgesic effect or a nonspecific effect on the animal's ability to respond (Hargreaves et al., 1988; Kowaluk et al., 2000). Additionally, the pattern of motor impairment produced by AK inhibitors and ADO receptor agonists is somewhat different than that observed for other classical CNS sedatives like benzodiazepines, barbiturates and ethanol where the dose–response curves for impairment of rotorod performance are either left of, or superimposed on, the dose–response curves for impairment of exploratory motor activity (unpublished observations). Thus, AK inhibitors and ADO receptor agonists appear to affect aspects of motor performance and coordination that are distinctly different that those produced by classical CNS sedatives. These findings are consistent with other data indicating that AK inhibitor-mediated antinociception is primarily mediated by actions at spinal sites whereas impairment of locomotor activity is mediated via supraspinal mechanisms (McGaraughty et al., 2001a; Suzuki et al., 2001; Zhu et al., 2001).

Taken together, the present data demonstrate that, unlike ADO receptor agonists, AK inhibitors potently attenuate inflammatory thermal hyperalgesia at doses that do not significantly alter motor performance. Since higher systemic doses of AK inhibitors and all antinociceptive doses of ADO receptor agonists impair some aspects of motor performance, the potential for motor impairment must be carefully considered in the analysis of the ability of ADO to reduce acute and chronic nociception. The present data also demonstrate that the pattern of motor impairment produced by activation of ADO receptors may be phenotypically distinct from that produced by classical sedative agents like benzodiazepines and barbiturates. While AK inhibition offers an improved therapeutic window (analgesia vs. motor performance) as compared to direct-acting ADO receptor agonists, additional work is necessary to fully characterize the side-effect profile of novel AK inhibitors.

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