Effects of A-134974, a Novel Adenosine Kinase Inhibitor, on Carrageenan-Induced Inflammatory Hyperalgesia and Locomotor Activity in Rats: Evaluation of the Sites of Action

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ABSTRACT

The present study investigated 1) antihyperalgesic actions of a novel and selective adenosine kinase (AK) inhibitor, A-134974 (IC_{50} = 60 pM), in the carrageenan model of thermal hyperalgesia; 2) effects of A-134974 on locomotor activity; and 3) relative contributions of supraspinal, spinal, and peripheral sites to the actions of A-134974. Systemic A-134974 (i.p.) dose dependently reduced hyperalgesia (ED_{50} = 1 μmol/kg) and at higher doses, reduced locomotor activity (ED_{50} = 16 μmol/kg). Administration of A-134974 intrathecally (i.t.) was more potent (ED_{50} = 6 nmol) at producing antihyperalgesia than delivering the compound by intracerebroventricular (ED_{50} = 100 nmol, i.c.v.) or intraplantar (ED_{50} >300 nmol) routes. In contrast, i.c.v. administration of A-134974 was more effective in reducing locomotor activity than i.t. administration (ED_{50} values were 1 and >100 nmol, respectively). Increasing the pretreatment time for i.t.-delivered A-134974 caused a greater reduction in locomotor activity (ED_{50} = 10 nmol). This was due to diffusion of A-134974 (i.t.) to supraspinal sites. The antihyperalgesic effects of systemic A-134974 were antagonized by the adenosine receptor antagonist theophylline (THEO, 30–500 nmol) administered i.t., but not i.c.v. In the locomotor assay, i.t.-injected THEO did not antagonize hypomobility caused by systemic or i.t. administration of A-134974. However, i.c.v. infusion of THEO did block the hypomotive actions of i.c.v.-, i.t.-, and i.p.-administered A-134974. These data demonstrate that the novel AK inhibitor A-134974 potently reduces thermal hyperalgesia primarily through interactions with spinal sites, whereas its ability to depress locomotor activity is predominantly mediated by supraspinal sites.

Adenosine (ADO) acts as an inhibitory neuromodulator throughout the central and peripheral nervous systems (Williams and Jarvis, 2000). Its activity is mediated via interactions with four different ADO receptor subtypes, A_{1}, A_{2A}, A_{2B}, and A_{3}, which are widely distributed in the brain, spinal cord, and peripheral tissues (Geiger et al., 1984; Choca et al., 1988; Sawynok, 1998; Moreau and Huber, 1999). Administration of ADO or ADO receptor agonists has been shown to attenuate nocifensive behaviors in both humans and animals (Sollevi, 1997; Sawynok, 1998). Mirroring the distribution of ADO receptors, brain (Herrick-Davis et al., 1989), spinal (Lee and Yaksh, 1996; Poon and Sawynok, 1998), and peripheral (Karlsten et al., 1992; Sawynok et al., 1998) sites of action have been implicated in ADO-induced antinociception. Although it may be beneficial to modulate nociception at diverse loci, the widespread distribution of ADO receptors also increases the likelihood of nonspecific ADO actions affecting such endpoints as the cardiovascular (Belardinelli et al., 1989) and psychomotor systems (Jarvis, 1997).

Inhibition of an ADO-metabolizing enzyme, adenosine kinase (AK), may represent a mechanism to minimize nonspecific effects of ADO. AK inhibition raises extracellular ADO concentrations (Davies et al., 1984, 1986) and increases endogenous ADO release (Pak et al., 1994; Golembiowska et al., 1996). One such AK inhibitor, 5′-deoxy,5′-iodotubercidin (5′d-5IT), has been demonstrated to selectively increase endogenous ADO levels in traumatized tissue (Britton et al., 1999). Increasing endogenous ADO concentrations through this mechanism may advantageously limit ADO activity to stressed biological regions or systems (Engler, 1987; Mullane and Young, 1993). Indeed, administration of the AK inhibitor GP683 lowered the levels of required desflurane anesthesia in dogs without producing the typical adverse cardiovascular effects often associated with direct-acting ADO receptor agonists (Wang et al., 1997).

AK inhibitors such as 5′d-5IT, 5′-amino-5′-deoxyADO (NH_{4}dADO), and 5-iodotubercidin (5IT) have demonstrated antinociceptive activity in a diverse array of nociceptive models. Systemic delivery of these AK inhibitors alleviated acute thermal nociception (mouse hot-plate) through nonopioid mechanisms.
Intrathecal (i.t.) infusion of NH₂dADO has also been shown to alleviate acute thermal nociception (Keil and DeLander, 1994). In the formalin model of persistent pain, intrathecal or intraplantar injection of NH₂dADO produced antinociception (Poon and Sawynok, 1995; Sawynok et al., 1998). Furthermore, in the carrageenan model of inflammatory pain, intrathecally administered 5IT and NH₂dADO were antihyperalgesic (Poon and Sawynok, 1995; Sawynok et al., 1998). Unlike direct-acting agonists (CGS 21680 and N⁶-cyclohexyladenosine), 5IT and NH₂dADO (i.t.) did not produce noticeable motor impairment, strengthening the utility of AK inhibitors as potential analgesics for animals in pathological nociceptive states.

In an effort to further evaluate and understand the antinociceptive activity of AK inhibitors, the present series of experiments 1) investigated antihyperalgesic actions of a novel AK inhibitor, A-134974 (Fig. 1), in the carrageenan model of inflammatory hyperalgesia; 2) measured the effects of this compound on locomotor activity; and 3) explored the relative contributions of supraspinal, spinal, and peripheral ADO sites of action to these behaviors. A-134974 is a structurally novel AK inhibitor that has been previously reported to reduce brain infarct size in the middle cerebral artery occlusion model of transient ischemia (Kowaluk et al., 1997).

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats (260–320 g; Charles River, Wilmington, MA) were housed in a temperature-controlled room with a 12/12-h day/night cycle. Following surgical procedures, animals were housed one per cage with food and water available ad libitum. All animal handling and experimental protocols were approved by Abbott’s Institutional Animal Care and Use Committee, and were conducted in accordance with the ethical principles for pain-related animal research of the American Pain Society.

Implantation of Intrathecal Catheters

For spinal drug administration, animals were implanted with chronic indwelling catheters. Under halothane inhalation anesthesia, PE-5 catheters (external PE-10; Marsil Enterprises, San Diego, CA) were inserted through the cisternal membrane at the base of the skull. All animals were given at least 7 days to recover from surgery before being tested. Placements were histologically verified. Additionally, under pentobarbital anesthesia (60 mg/kg i.p.) some animals were implanted with both an intrathecal catheter and a ventricular cannula. The catheter was placed first.

Drug Administration Procedures

Drugs administered to rats were A-134974 (Cowart, 1997, an AK inhibitor synthesized at Abbott Laboratories), morphine sulfate (Mallinckrodt, St. Louis, MO), and theophylline (a nonselective ADO receptor antagonist; Sigma Chemical Co., St. Louis, MO). All drugs were dissolved in sterile water for local (intraplantar, i.t., or i.c.v.) or systemic (i.p.) delivery. The drug administration procedures described below were followed for locomotor activity experiments as well as for hyperalgesia experiments. Each experimental group consisted of at least five animals.

Systemic Administration of A-134974. A-134974 (0.3–30 μmol/kg) or vehicle was administered i.p. 30 min before testing. Morphine was injected (i.p., 30-min pretreat) into animals in hyperalgesia experiments only (1.5–6 μmol/kg).

Local Administration of A-134974. A-134974 (3–100 nmol) or vehicle was injected directly into 1) the lumbar spinal cord via indwelling intrathecal catheters, 2) the right lateral ventricle, or 3) the intraplantar region of a carrageenan-inflamed hindpaw (hyperalgesia experiments only). A-134974 was administered into one of these regions at two different time points: 5 or 30 min prior to testing. Intrathecal injections were done over a 2-min period. The volume of injection was 10 μl followed by a 10 μl sterile water flush. Ventricular infusions occurred over a 4-min period with a total volume of 5 μl. In carrageenan-induced inflammatory hyperalgesia experiments, A-134974 was injected into the inflamed (right) hindpaw in a volume of 100 μl. To explore possible systemic actions of the drug following this intraplantar route of administration, the left, noninflamed paw was also injected with A-134974 or vehicle.

Antagonism of Local Activity. Theophylline (30–500 nmol) was administered i.t., i.c.v., or into the intraplantar region (inflamed and noninflamed hindpaw) to antagonize an effective locally administered dose of A-134974 (10–300 nmol). A-134974 and theophylline were injected 30 and 5 min, respectively, prior to testing. Specific injection rates and volumes were the same as described above. Appropriate controls were included in these experiments.

Antagonism of Systemic Activity. To investigate the site(s) of action following systemic delivery, an effective systemic dose of A-134974 (3–10 μmol/kg i.p.) or vehicle was given 30 min before testing. Theophylline or vehicle was then administered locally (intraplantar, i.t., or i.c.v.) 5 min before testing.

Behavioral Testing

Carrageenan-Induced Thermal Hyperalgesia. Using the model described by Hargreaves et al. (1988), the plantar surface of the right hindpaw in each rat was injected with 1 mg of carrageenan (in 100 μl of saline). Immediately following the injection of carrageenan, animals were placed in plastic chambers (18 × 29 × 12.5 cm) resting on a temperature-regulated (30°C) glass surface. The animals were removed from these chambers for drug or vehicle administration as outlined above. After each injection, the animals were returned to their respective chambers (the glass surface was cleaned each time). Two hours after carrageenan injection, animals were tested for thermal hyperalgesia. Briefly, through the glass surface, a radiant heat source (5-V, 50-watt projector bulb) was focused onto the plantar surface of the hindpaw. The rat’s withdrawal latency to this stimulus was recorded to the nearest 0.1 s. Each animal’s latency score was an average of two trials, which were separated by at least 5 min. The left hindpaw was not injected with carrageenan but was similarly tested allowing direct comparisons between inflated

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**Fig. 1.** Structure of A-134974.
and noninflamed paws for each animal. Withdrawal latencies after injection into a hindpaw did not differ from animals receiving no injection (S. McGaraughty, unpublished observations).

**Locomotor Activity.** Following drug or vehicle administration, rats were placed in an open testing chamber (42 × 42 × 30 cm) for 30 min. The chambers were located in a ventilated room with no noise attenuation. During the 30-min testing period, the animal's horizontal beam interruptions were recorded with a Digiscan Animal Activity Monitor (16 beam, 1-inch resolution; AccuScan Instruments, Columbus, OH). Locomotor activity was defined as the total number of horizontal beam interruptions over 30 min.

**Data Analysis of Behavioral Experiments**

**Carrageenan-Induced Hyperalgesia.** Withdrawal latencies were recorded from both the inflamed and noninflamed hindpaws. Means were compared within groups (inflamed versus noninflamed paws) and between drug/vehicle-injected groups. “Reversal in hyperalgesia” scores for each animal were calculated by the following formula: (latency inflamed paw/vehicle group − mean latency noninflamed paw/vehicle group)/(mean latency inflamed paw/vehicle group) × 100. In cases of negative values, the scores were designated as 0 (no reversal in hyperalgesia). Statistical significance was established by an ANOVA and a Fisher's protected least-significant difference post hoc analysis (p < 0.05).

**Locomotor Activity.** Total number of horizontal beam interruptions was counted over a 30-min period for each animal. Each of these values was then expressed as a percentage of the mean score obtained from the vehicle-injected control group. Statistical significance on group means was measured by an ANOVA followed by a Fisher's protected least-significant difference post hoc analysis (p < 0.05). ED50 values for all hyperalgesia and locomotor experiments were estimated using linear regression.

**In Vitro Assays**

AK enzyme inhibition was assayed radiochemically as described by Yamada et al. (1980) and McNally et al. (1997). The ability of A-134974 to inhibit AK activity in intact IMR-32 neuroblastoma cells (American Type Culture Collection, Gaithersburg, MD) carried out as previously described (Kowaluk and Cowart, 1994). Radioligand binding assay methodology for the A1, A2A, and A3 receptors was carried out as described by Jarvis et al. (2000). The ability of A-134974 to inhibit [3H]nitrobenzylthioinosine binding to the ADO transporter and to inhibit adenosine deaminase activity was also examined using previously described methodology (Parkinson and Geiger, 1996).

**Results**

A-134974 is a potent (IC50 = 60 pM) AK inhibitor (Table 1). It is highly selective for AK compared with other sites of ADO action, including A1, A2A, and A3 receptors and the ADO deaminase enzyme.

**Systemic Actions of A-134974**

In hyperalgesia experiments, 2 h after the injection of carrageenan into the right hindpaw, the paw was red and swollen. The contralateral, left, hindpaw appeared unaffected. In control group rats (receiving a systemic injection of vehicle, i.p.), withdrawal latencies after radiant heat stimulation of inflamed paws (2.83 ± 0.28 s) were significantly shorter (p < 0.01) than noninflamed paws (10.24 ± 0.17 s), indicating a carrageenan-induced hyperalgesia. A-134974 (i.p.) dose dependently reversed carrageenan-induced hyperalgesia (Fig. 2A) with an ED50 of 1 μmol/kg. For comparison, the antihyperalgesia ED50 following morphine injection (i.p.) was 3 μmol/kg. At the doses tested, A-134974 (i.p.) did not

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\text{TABLE 1} \\
\text{Selectivity of A-134974 for AK versus other sites of ADO interaction} \\
\text{Values represent means ± S.E.M. from at least three individual experiments. AK (intact cells) represents ADO phosphorylation in intact IMR-32 cells as previously described (Jarvis et al., 2000).} \\
\begin{array}{|c|c|} 
\hline 
\text{Site} & \text{IC50 (nM)} \\
\hline 
\text{AK} & 0.06 ± 0.07 \\
\text{AK (intact cells)} & 45 ± 8.6 \\
\text{ADO A1 receptor} & >100,000 \\
\text{ADO A2A receptor} & >100,000 \\
\text{ADO A3 receptor} & >100,000 \\
\text{ADO deaminase} & >100,000 \\
\text{ADO transporter} & >100,000 \\
\hline 
\end{array} \\
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**Fig. 2.** Effects of systemic (i.p.) A-134974 on thermal hyperalgesia and locomotor activity. In both A and B, the compounds were administered 30 min before testing commenced. In A, mean “antihyperalgesic” scores following stimulation of inflamed paws are shown; a score of 100% indicates that there was a complete compound-related reversal of hyperalgesia. Both morphine (i.p., n = 6) and A-134974 (n = 6) reversed carrageenan-induced inflammatory hyperalgesia. Latencies of noninflamed hindpaws were unaffected by administration of A-134974 (data not shown), compared with latencies of vehicle-injected animals (points shown at 100%). In B, A-134974 dose dependently decreased locomotor activity (n = 19–20). *p < 0.05, **p < 0.01 versus vehicle control group, values are ±S.E.M.
significantly alter withdrawal latencies of the noninflamed paws.

In the locomotor assay, systemic (i.p.) administration of A-134974 significantly (p < 0.01) and dose dependently depressed rat locomotor activity, which was measured by the number of beam interruptions over a 30-min period (ED₅₀ = 16 μmol/kg, Fig. 2B). No overt signs of limb impairment were apparent. The number of beam interruptions for the vehicle-injected group was 5277 ± 327.

Site-Specific Effects of A-134974 on Carrageenan-Induced Hyperalgesia

**Spinal Activity.** In animals with i.t. catheters, significant carrageenan-induced hyperalgesia (p < 0.01) was observed in the vehicle-injected groups at both pretreatment times. With a 5-min pretreatment of vehicle (i.t.), withdrawal latencies were 3.4 ± 0.36 and 10.77 ± 0.57 s for inflamed and noninflamed paws, respectively. Latencies after a 30-min pretreatment were 3.5 ± 0.27 s (inflamed paw) and 9.43 ± 0.49 s (noninflamed paw). These latencies were similar to the values obtained from animals without surgery, demonstrating that indwelling i.t. catheters and subsequent i.t. administration of vehicle did not interfere with hindlimb withdrawal from noxious heat. The i.t. administration of A-134974 (Fig. 3A), at either pretreatment time, caused significant antihyperalgesia (p < 0.01) without altering the withdrawal latencies of the noninflamed hindpaws. The antihyperalgesia ED₅₀ values were 6 nmol (5 min) and 2 nmol (30 min).

Theophylline, administered i.t. (100 and 500 nmol), antagonized the potent antihyperalgesic activity of systemic A-134974 (3 μmol/kg i.p.), implicating spinal sites of action for A-134974 following systemic delivery (Fig. 3B). Intrathecral administration of theophylline (30 and 100 nmol) also antagonized the antihyperalgesic effects of A-134974 (30 nmol) injected i.t. (Fig. 3C). Moreover, in this latter experiment, 100 nmol of theophylline (i.t.) completely reversed the local spinal effects of A-134974. Administration of theophylline alone (i.t., 100 and 500 nmol) did not affect withdrawal latencies after stimulation of either hindpaws. The strong antihyperalgesia observed following i.t. injection of A-134974 was not due to diffusion of the compound to supraspinal sites since this effect could not be antagonized by i.c.v. infusion of theophylline (Fig. 3D).

**Supraspinal Activity.** At both pretreatment times (5 and 30 min), withdrawal latencies of the inflamed paws from animals implanted with ventricular cannulae were signifi-

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**Fig. 3.** Spinal effects on carrageenan-induced inflammatory hyperalgesia. Score of 100% indicates complete reversal of hyperalgesia. A, dose-dependent actions of A-134974 injected i.t. 5 or 30 min before testing (n = 6–18). A-134974 did not significantly alter latencies of the noninflamed hindpaws (data not shown). B through D, A-134974 and theophylline were injected 30 and 5 min, respectively, before testing. Intrathecally administered theophylline (30, 100, and 500 nmol) antagonized both systemic- (B) and spinal (C)-related antihyperalgesic activity of A-134974 (n = 6). D, i.c.v.-infused theophylline did not antagonize the effects of i.t.-injected A-134974 (n = 6). **p < 0.01 versus vehicle control group, ***p < 0.01 versus respective A-134974-vehicle group. Values are expressed as ±S.E.M.
cantly lower than the noninflamed hindpaws (p < 0.01), indicating the presence of thermal hyperalgesia. The withdrawal latencies in the vehicle group at 5-min pretreat were 3.04 ± 0.26 s (inflamed) and 10.82 ± 0.53 s (noninflamed), whereas at 30-min pretreat the latencies were 2.45 ± 0.17 s (inflamed) and 10.1 ± 0.44 s (noninflamed). A relatively weak, but significant antihyperalgesia was observed following i.c.v. infusions of A-134974 (Fig. 4A). The ED_{50} values were 100 nmol (5-min pretreat) and 60 nmol (30-min pretreat). Withdrawal latencies of the noninflamed hindpaws were unaffected by i.c.v. administration of A-134974.

Administration of theophylline (500 nmol) into the right lateral ventricle did not antagonize the antihyperalgesic effects of systemically injected A-134974 (3 μmol/kg i.p., Fig. 4B). These results demonstrate that supraspinal sites have a minimal contribution to the antihyperalgesic effects of systemically (i.p.) administered A-134974.

**Intraplantar Injections.** Withdrawal latencies following intraplantar injection of vehicle into the inflamed paw 5 min before testing were 4.26 ± 0.75 s (inflamed paw) and 10.2 ± 0.83 s (noninflamed paw, p < 0.01). With a 30-min pretreatment of vehicle, the latencies were 3.2 ± 0.33 s (inflamed) and 9.5 ± 0.49 s (noninflamed, p < 0.01). Thermal hyperalgesia was therefore observed in both control groups. A-134974 did not affect withdrawal latencies of either paw when injected into the inflamed paw 5 min before testing (ED_{50} of >300 nmol, Fig. 5A). Significant (p < 0.01) antihyperalgesia was observed after A-134974 was administered into the inflamed paw 30 min before testing (ED_{50} of 100 nmol). However, injection of 300 nmol of A-134974 into the contralateral noninflamed paw at this 30-min pretreatment time also caused a significant antihyperalgesic rise in withdrawal latencies of the inflamed paw (p < 0.05). This effect on the inflamed hindpaw latency following injection of A-134974 into the contralateral hindpaw suggests that the compound may have distributed systemically after this route of injection.

Direct administration of theophylline into the carrageenan-inflamed paw (100 and 500 nmol) antagonized the antihyperalgesic activity of systemic A-134974 (3 μmol/kg, i.p.). However, intraplantar injection of theophylline (500 nmol) into the contralateral noninflamed hindpaw also antagonized the antihyperalgesic action of systemic A-134974 (3 μmol/kg i.p., Fig. 5B). Therefore, the antagonism following the intraplantar administration of theophylline was not site-specific, and was likely due to systemic diffusion of theophylline itself. Antihyperalgesia caused by direct administration of A-134974 into the inflamed paw was antagonized by local injection of theophylline into that same inflamed hindpaw (Fig. 5C). Theophylline administered into the contralateral noninflamed hindpaw did not reverse this local antihyperalgesic action of A-134974.

**Site-Specific Effects of A-134974 on Locomotor Activity**

**Spinal Activity.** Animals with indwelling i.t. catheters, despite no overt signs of limb impairment or illness, were less active when placed in the locomotor activity boxes compared with animals without surgical treatment or animals implanted with ventricular cannulae. Animals injected with vehicle (i.t.), tallied 2687 ± 294 (5-min pretreatment) and 4896 ± 610 (30-min pretreatment) beam interruptions over a 30-min period in the locomotor activity assay. Intrathecal administration of A-134974, 30 min before testing, significantly (p < 0.01) depressed locomotor activity with an ED_{50} of 3 nmol/kg i.p. (5-min pretreatment) or i.t. (30 nmol)-injected A-134974. This lack of antagonism by i.t. theophylline indicated that the depressive effects of A-134974 on locomotor activity might not be due to an interaction with spinal mechanisms. Hypomobility following i.t. administration of A-134974 (30-min pretreat) might therefore be due to diffusion of the compound to sites outside the spinal cord. Diffusion of A-134974 (i.t.) from spinal to supraspinal sites was investigated by administering theophylline directly into the right lateral ventricle (i.c.v.). Indeed, 500 nmol of theophylline infused i.c.v. significantly reversed
hypomobility caused by i.t. injection of 100 nmol of A-134974 (Fig. 6D).

**Supraspinal Activity.** Direct i.c.v. administration of A-134974 significantly ($p < 0.01$) decreased spontaneous locomotor activity at both pretreatment times (vehicle group scores at 5- and 30-min pretreat were 7757 ± 1093 and 8799 ± 896 beam interruptions, respectively; Fig. 7A). ED$_{50}$ values for A-134974 in this assay were 1 nmol (5 min) and 4 nmol (30 min). No signs of limb paralysis were evident at the doses tested (even up to 100 nmol; data not shown).

As shown in Fig. 7 (B and C), systemic (10 μmol/kg i.p.) or supraspinal (10 nmol i.c.v.) delivery of A-134974 decreased locomotor activity by more than 75% from the respective control groups. These effects of A-134974 were reversed by i.c.v. infusion of 500 nmol of theophylline. This dose of theophylline by itself (i.c.v.) did not significantly affect exploratory activity (Figs. 6C and 7, B and C).

To address the possibility that the hypomobility observed after this i.c.v. infusion of A-134974 might be due to its diffusion into the spinal cord, an attempt was made to antagonize i.c.v. administered A-134974 with theophylline injected i.t. Theophylline (500 nmol) administered i.t. did not antagonize hypomotive actions of 10 nmol of A-13974 infused i.c.v. (Fig. 7D).

**Discussion**

Systemic delivery (i.p.) of the highly selective AK inhibitor A-134974 potently and dose dependently reversed carrageenan-induced thermal hyperalgesia. These effects were comparable to morphine’s antihyperalgesic actions (ED$_{50}$ values of 1 and 3 μmol/kg, respectively). The analgesic effect of A-134974 was selective for the inflamed but not the noninflamed hindpaw, a result consistent with the proposed beneficial effects of increased ADO concentrations at sites of injury or trauma (Engler, 1987; Mullane and Young, 1993). The antihyperalgesic actions of systemic A-134974 were also separable from the compound’s hypomotive actions. A 16-fold separation between ED$_{50}$ values for hypomotive and antihyperalgesic actions of systemic A-134974 was observed. Furthermore, in the hyperalgesia experiments, withdrawal latencies of the noninflamed hindpaws did not differ between groups receiving either A-134974 or vehicle, demonstrating that the administration of A-134974 did not impair withdrawal reflexes.

The antihyperalgesic activity of systemically administered A-134974 was most likely due to interactions with both central and peripheral mechanisms. However, contributions from supraspinal sites appear relatively small compared with the antihyperalgesic contribution of spinal sites. Site-specific antagonism of the systemic effects of A-134974 was seen after theophylline administration into the spinal cord but not the brain. Nonetheless, antihyperalgesia was shown after direct infusion of A-134974 into the lateral ventricles; however, the ED$_{50}$ was approximately 16-fold greater than the value measured after intrathecal infusion (5-min pretreatment). The supraspinal potency of A-134974 in revers-
ing hyperalgesia was thus relatively weak. Direct-acting ADO receptor agonists have also shown improved antinociceptive efficacy after injection into the spinal cord compared with delivery into the brain (Holmgren et al., 1986). Nonetheless, Herrick-Davis et al. (1989) have reported potent antinociception following supraspinal administration of ADO agonists, but, this effect could not be separated from drug-induced sedation/ataxia.

Direct injections of theophylline into carrageenan-inflamed hindpaws or into the spinal cord similarly antagonized systemic antihyperalgesic actions of A-134974. This might suggest that mechanisms at both sites are important to the systemic action of A-134974. However, theophylline administered into the noninflamed hindpaw also antagonized the antihyperalgesic effects of systemic A-134974. This nonspecific antagonism by theophylline obscures any conclusions regarding peripheral sites of action for systemically administered A-134974. Nonetheless, contributions from mechanisms local to the inflamed hindpaw cannot be dismissed. Sawynok et al. (1998) have demonstrated that coadministration of the AK inhibitor NH2dADO with low concentrations of formalin into a hindpaw reduced formalin-induced nociceptive responses. Furthermore, injection of NH2dADO into the hindpaw contralateral to the formalin injection was ineffective, ruling out a possible systemic action. In the present study, direct intraplantar injection of A-134974 into the inflamed hindpaw also resulted in antihyperalgesia. However, at either pretreatment time (5 and 30 min), this hindpaw effect of A-134974 (ED50 values were >300 nmol and 100 nmol) was much less potent than the antihyperalgesic activity observed after spinal administration of the compound (ED50 values were 6 and 2 nmol, respectively). Taken together, the hyperalgesia experiments demonstrate that although supraspinal and peripheral sites may be involved, spinal sites were the major contributors to the antihyperalgesic effects of systemically administered A-134974.

ADO receptors are found in both the dorsal and ventral horns of the spinal cord (Geiger et al., 1984; Choca et al., 1988). Through binding at these sites, ADO may modulate, respectively, both the nociceptive and spinal motor systems. Intrathecal delivery of ADO receptor agonists has been shown to impair spinal motor function at doses greater than those producing antinociception (Karlsten et al., 1990; Lee and Yaksh, 1996). A similar finding was observed following intrathecal administration of A-134974 at the 5-min pretreatment time. There was at least a 16-fold separation between ED50 values in the locomotor (>100 nmol) and hyperalgesia assays (6 nmol) after infusion of A-134974 (i.t.). However, at the 30-min pretreatment time, intrathecal administration of A-134974 caused a significant reduction in locomotor activity (ED50 was 10 nmol). Nonetheless, despite a strong depressant effect following spinal administration of

![Fig. 6. Spinal effects on locomotor activity. A, dose-dependent actions of A-134974 injected i.t. 5 min (n = 15–16) or 30 min (n = 6–12) before testing. B through D, A-134974 and theophylline were injected 30 and 5 min, respectively, before testing. Theophylline, injected i.t., did not antagonize the hypomotive actions of i.p.- (B) or i.t. (C)-administered A-134974 (n = 6–7). However, in D, the depressant action of i.t.-injected A-134974 was antagonized by theophylline infused i.c.v. (n = 8). *p < 0.05, **p < 0.01 versus vehicle control group, p < .05 versus respective A-134974-vehicle group. Values are ±S.E.M.]
A-134974 at this pretreatment time, the site of A-134974 action on locomotor activity does not appear to be the spinal cord since the hypomotile effect of intrathecal A-134974 was not antagonized by intrathecal administration of theophylline. Hypomobility after intrathecal delivery of A-134974, at the 30-min pretreatment time, was likely due to diffusion of A-134974 to supraspinal sites since this effect was reversed by direct administration of theophylline into the lateral ventricles. Additionally, the disparity in ED50 values measured after the two different pretreatment times may reflect this diffusion. The longer period of time between injection and testing (30 versus 5 min) allowed an increased distribution of A-134974 to areas beyond the spinal lumbar region, which, most likely, included supraspinal sites. Clearly, spinal mechanisms played only minor role in depressing locomotor activity after the intrathecal administration of A-134974. On the other hand, the potent antihyperalgesic effect observed after intrathecal delivery of A-134974 was less likely a consequence of diffusion to sites away from the lumbar region since 1) as outlined above, A-134974 demonstrated relatively weak antihyperalgesic activity after direct administration into the lateral ventricles or hindpaw; 2) injection of theophylline into the lateral ventricles failed to antagonize this “spinal” antihyperalgesia; and 3) intrathecal theophylline successfully antagonized the antihyperalgesic action of intrathecal A-134974.

Direct administration of A-134974 into the lateral ventricles significantly depressed locomotor activity (ED50 of 1–4 nmol). The exact supraspinal site(s) causing hypomobility was not determined although ADO receptor agonists have demonstrated sedative/ataxic effects after injection into the nucleus accumbens, caudate-putamen, and striatal tissue (Barraco et al., 1994; Ferre et al., 1997; Hauber and Munkle, 1997). Infusion of theophylline into the lateral ventricles reversed the hypomotive action of systemic A-134974 (n = 4–6). **p < 0.01 versus vehicle control group; *p < 0.05 versus respective A-134974-vehicle group. Values are ±S.E.M.
pomobility would be difficult to interpret. This was not the case in the present experiments; ventricular injection of theophylline at the highest dose tested, 500 nmol, did not significantly increase locomotor activity.

In conclusion, the novel AK inhibitor A-134974 potently reverses carrageenan-induced inflammatory hyperalgesia through interactions with central and peripheral sites, although spinal sites of action are the primary contributors to this effect. These antihyperalgesic actions of A-134974 are separable from ataxic/sedative properties of the compound, which are predominantly mediated by supraspinal sites. A-134974 may be a useful tool to further explore the therapeutic use of AK inhibitors as analgesic agents.

References

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Sites of Action for a Novel Adenosine Kinase Inhibitor 509

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