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An adenosine kinase inhibitor attenuates tactile allodynia in a rat model of diabetic neuropathic pain

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Abstract

The present study was conducted to characterize the development of tactile allodynia in the streptozotocin-induced rat model of diabetes, and to evaluate the antinociceptive effects of systemically administered morphine and the adenosine kinase inhibitor, 5′-deoxy-5-iodotubercidin (5′d-5IT) in this model. Rats were injected with 75 mg/kg streptozotocin (i.p.), and blood glucose levels were determined 3–4 weeks later. Diabetic (blood glucose levels $\geq$ 250 mg/dl) and vehicle-injected rats were examined weekly for the development of tactile allodynia by measuring the threshold for hind paw withdrawal using von Frey hairs. Withdrawal thresholds were reduced to 6.8 $\pm$ 0.6 g (mean $\pm$ S.E.M.) in approximately one-third of streptozotocin-treated rats 7 weeks after streptozotocin treatment as compared to control thresholds (13.2 $\pm$ 0.1 g), and this allodynia persisted for at least an additional 7 weeks. In additional experiments, morphine sulfate (5–21 μmol/kg, i.p.) produced dose-dependent antinociceptive effects on tactile allodynia for up to 2 h post-dosing. The adenosine kinase inhibitor, 5′d-5IT (2.5 and 5 μmol/kg, i.p.) also dose-dependently attenuated tactile allodynia. Pretreatment with the opioid receptor antagonist, naloxone (27 μmol/kg, i.p.) or the non-selective adenosine receptor antagonist, theophylline (111 μmol/kg, i.p.) significantly diminished the anti-allodynic effects of morphine and 5′d-5IT, respectively. The present study demonstrates that the potent and selective adenosine kinase inhibitor, 5′d-5IT, is equally effective as morphine in blocking tactile allodynia in this model.

Keywords: Allodynia; Adenosine kinase; Adenosine; Streptozotocin; Diabetes; Pain

1. Introduction

Neuropathic pain is generally considered to be one of the most common and troublesome complications afflicting diabetic patients, and diabetic neuropathic pain is amongst the more frequently encountered neuropathic pain syndromes (Vinik et al., 1992; Clark and Lee, 1995). Diabetic neuropathy is often characterized as pain, sometimes severe, that occurs mainly in the lower extremities (Ellenberg, 1976; Morley et al., 1984). Such pain can occur either spontaneously, as a result of exposure to normally mildly painful stimuli (i.e., hyperalgesia) or to stimuli not normally perceived as painful (i.e., allodynia) (Brown and Asbury, 1984). Tactile allodynia (the perception of touch as painful) represents one of the most troublesome complaints, given the inevitability of contact with the physical environment. Current drug therapy for painful neuropathy includes use of tricyclic antidepressants, topical capsaicin, non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants and opioid receptor agonists such as morphine. However, such analgesic relief is often inadequate, potentially toxic, or is associated with dependence liabilities (Arner and Meyerson, 1988; Clark and Lee, 1995).

The streptozotocin-treated rat has been widely used as a model of insulin-dependent diabetes mellitus, and a number of anomalies in pain perception have been demonstrated in this model (Hounsom and Tomlinson, 1997). For example, formalin-evoked flinching is exaggerated in streptozotocin-treated rats as compared to control animals (Calcutt et al., 1995, 1996). Mechanical hyperalgesia and thermal allodynia have also been observed following streptozotocin treatment (Akunne and Soliman, 1987; Wuarin-Bierman et al., 1987; Ahlgren and Levine, 1993; Courteix et al., 1993). In addition, the development of tactile allodynia has recently been reported in this animal model of diabetes (Calcutt et al., 1996; Calcutt and Chaplan, 1997).

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The present study was conducted to further characterize the onset and time course of tactile allodynia in streptozotocin-treated rats, and to evaluate the antinociceptive effects of morphine and the potent and selective adenosine kinase inhibitor, 5′deoxy-5-iodotubercidin (5′d-5IT) (Davies et al., 1984, 1986) in this model of neuropathic pain. Morphine has been demonstrated to dose-dependently attenuate mechanical hyperalgesia in streptozotocin-injected diabetic rats (Courteix et al., 1994), and opioid therapy has demonstrated efficacy in neuropathic pain patients (Portenoy and Foley, 1986; Rowbotham et al., 1991; Zenz et al., 1992). Adenosine kinase is a key metabolizing enzyme for adenosine, and adenosine kinase inhibitors increase extracellular levels of adenosine locally at sites of tissue injury (Davies et al., 1984; Britton et al., 1996). Adenosine has well documented effects as an inhibitory neuromodulator acting at specific cell-surface receptors (P1 receptors) in the central and peripheral nervous systems, and as an endogenous modulator of antinociceptive pathways. Consistent with an antinociceptive role of adenosine, direct-acting adenosine receptor agonists have shown efficacy in animal models of acute (Post, 1984; Karlsten et al., 1991), persistent (Sawynok et al., 1991), and neuropathic pain (Sosnowski and Yaksh, 1989; Yamamoto and Yaksh, 1992; Lee and Yaksh, 1996). Initial studies with adenosine kinase inhibitors also demonstrate efficacy in animal pain models of acute nociceptive and inflammatory pain (Keil and DeLander, 1992; Poon and Sawynok, 1995; Kowaluk et al., 1998a,b). In addition, adenosine itself and adenosine receptor agonists have been reported to produce analgesia in human pain syndromes (Segerdahl et al., 1995; Sollevi et al., 1995).

2. Materials and methods

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were group-housed (4–5 animals per cage) in standard plastic cages with wood chip bedding. Bedding was changed daily for all animals to maintain sanitary conditions. In individual experiments, rats (150–350 g) were injected intraperitoneally in groups of 80–100 animals with 75 mg/kg streptozotocin (Sigma, St. Louis, MO) dissolved in 0.9% saline. Vehicle controls were injected with an equal volume of 0.9% saline. Streptozotocin solutions were freshly prepared due to the limited stability of the compound (Rakieten et al., 1963). Blood glucose levels were determined in all animals using an Encore Glucometer (Bayer, Elkhart, IN) from blood samples obtained by tail vein bleeds. Since elevated blood glucose levels appear to stabilize approximately 3 weeks post streptozotocin-treatment (Courteix et al., 1993), blood glucose levels were assessed 3–4 weeks following streptozotocin treatment. Rats with blood glucose levels ≥ 250 mg/dl (≥ 14 mM) were considered diabetic and used for further studies. All animal handling and experimental protocols were approved by an institutional animal care and use committee (IACUC).

Tactile allodynia thresholds were assessed in streptozotocin-treated and control rats by placing the animals on an elevated aluminum screen with a 1.27×1.27 cm^2 grid to provide access to the ventral side of the left hind paw. An inverted, clear plastic cage ($29 \times 18 \times 12$ cm^3, l × w × h) was placed over each rat, and the animals were allowed to acclimate to the test environment for 20 min prior to baseline testing. Tactile allodynia thresholds were determined using von Frey hairs (Stoelting, Wood Dale, IL) as described by Chaplan et al. (1994), with each rat tested 3 times per time point. Fifty percent threshold values were calculated according to the up–down method of Dixon (1980). In studies examining the effects of test compounds on tactile allodynia in streptozotocin-treated animals, only rats with pre-dosing thresholds of ≤ 8.0 g were utilized. A percent maximal protective effect value (% M.P.E.) was calculated for each dose at each pre-treatment time according to the formula: ([post-drug threshold] − [baseline threshold])/([maximum threshold] − [baseline threshold]) × 100%, where maximum threshold was equal to 15 g (Lee et al., 1995). Data were analyzed (GB-STAT, Dynamics Microsystems, Silver Spring, MD) by analysis of variance with the repeated measure of time, and protected *t*-tests were then performed. Statistical significance was determined at $P < 0.05$.

Morphine sulfate was obtained from Mallinckrodt (St. Louis, MO), while naloxone hydrochloride and theophylline were from Sigma. Synthesis of 5′d-5IT was done at Abbott Laboratories. All compounds were dissolved in a 0.9% saline solution and injected, i.p., in a 1 ml/kg volume. Preparation of the 5′d-5IT solutions required addition of HCl to produce an approximately 30 mM HCl solution in saline, and vehicle controls were treated in a similar manner.

3. Results

Three weeks following streptozotocin treatment, 81% of the rats ($n = 65$) exhibited blood glucose levels in the diabetic range (≥ 250 mg/dl). In this group of streptozotocin-treated diabetic rats, tactile allodynia was assessed weekly beginning at 3 weeks post-streptozotocin treatment, at which time 17% of the animals were determined to have a significant tactile allodynia response (mean 50% threshold values of ≤ 10 g) (data not shown). By 7 weeks post-streptozotocin, approximately one third of these 65 diabetic animals had threshold scores of ≤ 10 g. At the start of the experiment, the mean body weights of both the streptozotocin and vehicle treated rats was 317 ± 10 g, (mean ± S.E.M.). At 7 weeks post streptozotocin-treatment, the vehicle-treated rats weighed 508 ± 24 g and the

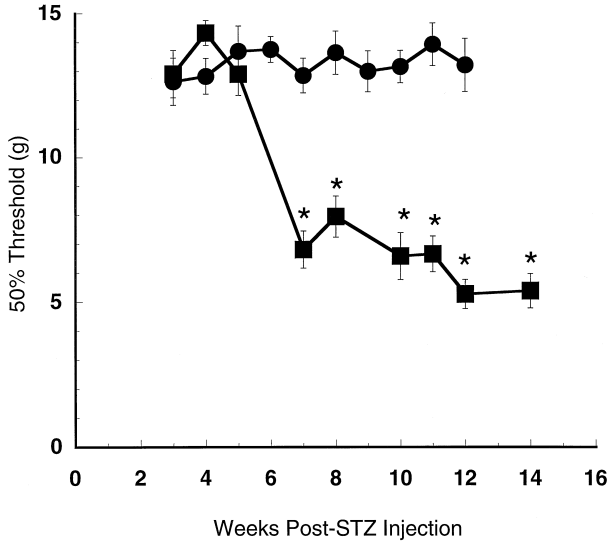

Fig. 1. Significant tactile allodynia developed in streptozotocin-treated diabetic rats (squares; $n = 19$) between 5 and 7 weeks post-streptozotocin injection that remained relatively stable for at least 14 weeks post-streptozotocin treatment. No significant difference for tactile threshold was found with the vehicle-injected control animals (circles; $n = 5$) during this same time period. $^{*}P < 0.05$ compared to week 3 tactile allodynia thresholds.

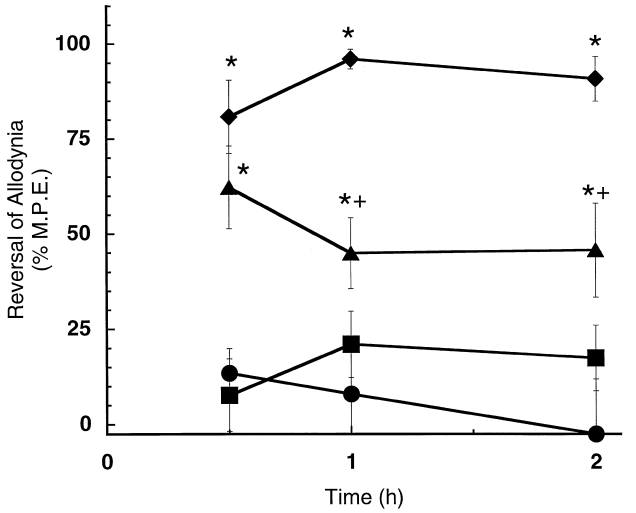

Fig. 3. Naloxone hydrochloride (27 μmol/kg, i.p., triangles) administered 30 min prior to injection of morphine sulfate (21 μmol/kg, i.p. diamonds) significantly reduced the antinociceptive effects of morphine sulfate. Naloxone treatment alone (squares) had no significant effect on tactile allodynia thresholds as compared to vehicle treated control animals (circles). $^{*}P < 0.05$ compared to vehicle-treated diabetic rats. $^{+}$ indicates $P < 0.05$ compared to the antinociceptive effects of morphine sulfate. $n = 6$ for all drug- and vehicle-treated groups. Baseline tactile thresholds assessed immediately prior to drug administration averaged 5.1 ± 0.3 g and were not significantly different between treatment groups.

streptozotocin-treated rats weighed 287 ± 8 g. Rats exhibiting streptozotocin-induced allodynia at week 7 were tested at weekly intervals, and found to have similar tactile allodynia thresholds for up to 14 weeks post-streptozotocin treatment (Fig. 1). Vehicle-treated control rats showed normal withdrawal thresholds (13.2 ± 0.1 g, mean ± S.E.M.) throughout this testing period (Fig. 1). Based on these results, subsequent experiments were conducted us-

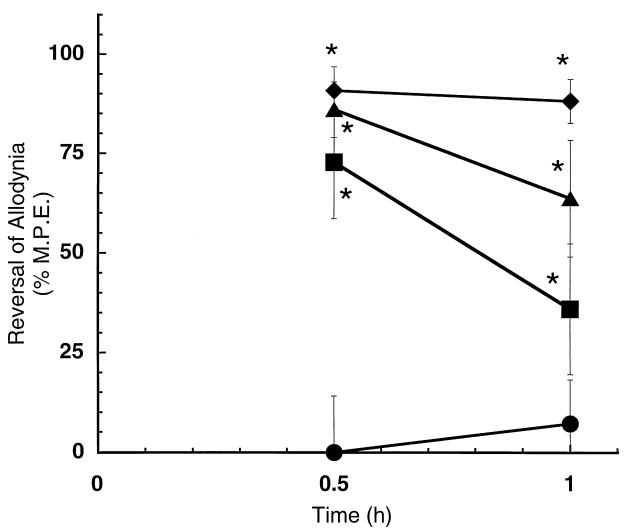

Fig. 2. The antinociceptive effects of morphine on tactile allodynia in streptozotocin-treated rats. Morphine sulfate produced a dose-dependent increase in tactile allodynia thresholds in diabetic rats (5 μmol/kg, i.p., squares; 10.2 μmol/kg, i.p., triangles; and 21 μmol/kg, i.p., diamonds) compared to vehicle-treated diabetic rats (circles) at 30 min and 1 h post-dosing. $^{*}P < 0.05$ compared to vehicle-treated diabetic rats. $n = 6$ for all drug- and vehicle-treated groups. Baseline tactile thresholds assessed immediately prior to drug administration averaged 5.6 ± 0.3 g and were not significantly different between treatment groups.

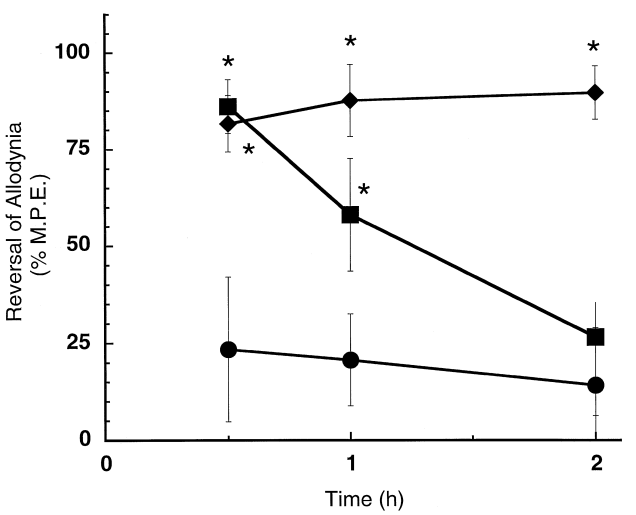

Fig. 4. The antinociceptive effects of 5′d-5IT on tactile allodynia in streptozotocin-treated rats. 5′d-5IT (2.5 μmol/kg, i.p., squares; 5 μmol/kg, i.p., diamonds) produced a dose-dependent antinociceptive effect on tactile allodynia in diabetic rats compared to vehicle-treated diabetic rats (circles) at 30 min to 2 h post-dosing. $^{*}P < 0.05$ compared to vehicle-treated diabetic rats. $n = 6$ for all drug- and vehicle-treated groups. Baseline tactile thresholds assessed immediately prior to drug administration averaged 5.5 ± 0.3 g and were not significantly different between treatment groups.

ing streptozotocin-treated diabetic rats from 7–14 weeks post-streptozotocin-treatment and with baseline tactile allodynia thresholds of ≤ 8.0 g.

Morphine dose-dependently attenuated tactile allodynia in streptozotocin-treated rats at 30 min and 1 h post-dosing (Fig. 2). Morphine (21 μmol/kg, i.p.) produced near-maximal efficacy at both time points examined. Naloxone (27 μmol/kg, i.p.) administered 30 min prior to morphine significantly attenuated the antinociceptive effects of morphine 1 and 2 h after dosing (Fig. 3). Tactile allodynia thresholds in diabetic rats that were pretreated with naloxone 30 min prior to injection of the vehicle control for morphine were not significantly different from those in diabetic rats treated with vehicle control for both injections.

The adenosine kinase inhibitor 5′d-5IT (2.5 and 5 μmol/kg, i.p.) also significantly attenuated tactile allodynia in the diabetic rats (Fig. 4). The higher dose of the adenosine kinase inhibitor produced a near-maximal antinociceptive effect, comparable to that of 21 μmol/kg, i.p., morphine sulfate, for up to 2 h post-dosing. The antinociceptive effect of 5 μmol/kg, i.p., 5′d-5IT was significantly reduced at all time points tested when diabetic rats were treated with the non-selective adenosine receptor antagonist theophylline (111 μmol/kg, i.p.) 30 min prior to 5′d-5IT injection (Fig. 5). This dose of theophylline alone had no statistically significant effect on tactile allodynia thresholds in the diabetic animals compared to vehicle controls ($n = 6$ animals per dosing group; vehicle = 13 ± 13% MPE and theophylline = 17 ± 13% MPE).

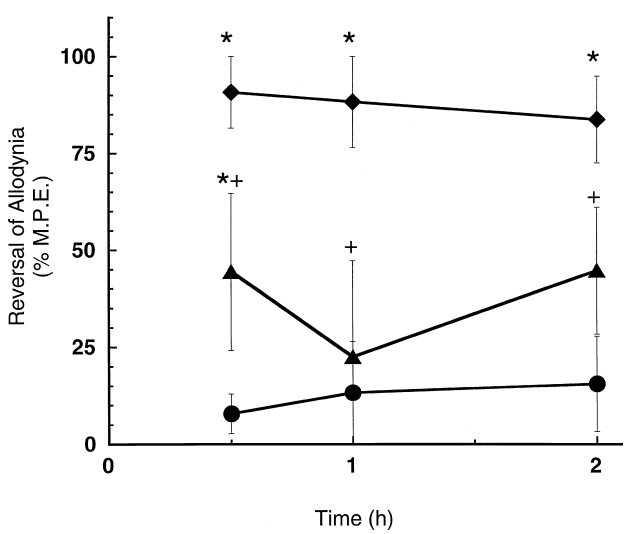

Fig. 5. The antinociceptive effect of 5′d-5IT (5 μmol/kg, i.p., diamonds) was reduced by pretreatment with theophylline (111 μmol/kg, i.p., triangles) 30 min prior to injection with 5′d-5IT as compared to vehicle-treated diabetic rats (circles). $^{*}P < 0.05$ compared to vehicle-treated diabetic rats. $^{+}P < 0.05$ compared to the antinociceptive effects of 5′d-5IT. $n = 6$ for all drug- and vehicle-treated groups. Baseline tactile thresholds assessed immediately prior to drug administration averaged 5.2 ± 0.3 g and were not significantly different between treatment groups.

4. Discussion

The present study describes the development of tactile allodynia in the streptozotocin-treated diabetic rat model. Tactile allodynia was evident in a subpopulation of rats at three weeks after streptozotocin treatment, and the population of rats exhibiting allodynic responses increased in number over the next four weeks. Streptozotocin-treated rats that exhibited significant tactile allodynia at 7 weeks post streptozotocin-treatment continued to show stable allodynic responses for at least 14 weeks post-streptozotocin injection.

The present results coupled with other recent reports (Calcutt et al., 1996; Calcutt and Chaplan, 1997) provide further evidence that streptozotocin-treated diabetic rats develop a variety of anomalies of pain perception, in particular allodynic responses to non-noxious tactile stimulation. These observations extend other reports of streptozotocin-induced alterations in nociception, including both thermal (Forman et al., 1986; Lee and McCarty, 1992) and mechanical hyperalgesia (Ahlgren and Levine, 1993; Zhuang et al., 1996), as well as increased pain responses in the formalin test of chemically-stimulated hyperalgesia (Courteix et al., 1993; Calcutt et al., 1995). Since altered patterns of nociception do not appear to be attributable to inherent streptozotocin neurotoxicity (Calcutt and Chaplan, 1997), streptozotocin-induced hyperglycemia clearly induces a variety of pathophysiological consequences that can lead to altered nociceptive responses (Hounsom and Tomlinson, 1997).

In the present study, streptozotocin treatment produced significant tactile allodynia that required several weeks to become evident in a subpopulation of treated rats. These results stand in contrast to a recent report indicating that tactile allodynia is expressed within days of streptozotocin treatment (Calcutt et al., 1996), becoming significant by approximately three weeks. Methodological differences between the two studies, including gender of the animals, housing conditions and experimental manipulations in the determination of nociceptive responses (Calcutt et al., 1996; Hounsom and Tomlinson, 1997) may have contributed to the observed differences in time to onset of allodynia. Importantly, in both studies, once allodynia had developed, it persisted for many weeks. While few detailed investigations of the time-course for the expression of streptozotocin-induced alterations in nociceptive responses have been reported, other data also suggest that there is some variability between studies in the temporal expression of altered nociception following streptozotocin-treatment. For example, the onset of mechanical hyperalgesia (decreased withdrawal latency to paw pressure) has been reported to range from one (Zhuang et al., 1996) to 3–4 weeks (Wuarin-Bierman et al., 1987; Courteix et al., 1993). Courteix et al. (1993) reported that streptozotocin-induced thermal allodynic responses were present as early as 2 weeks post-streptozotocin treatment in a subpopulation of

diabetic rats, and that this subpopulation increased in number with time following streptozotocin treatment. Similarly, streptozotocin-induced increases in nociception in the formalin test were found to develop over time requiring at least 4 weeks to fully develop (Courteix et al., 1993; Calcutt et al., 1995).

The current study demonstrated that, in rats that do develop tactile allodynia following streptozotocin treatment, systemically administered morphine, at doses as low as 5 μmol/kg, i.p., reduced tactile allodynia. A significant reduction in the anti-allodynic effects of morphine by pretreatment with naloxone indicated that this was an opioid receptor-mediated event. These results are consistent with a recent study showing that intrathecal morphine can attenuate tactile allodynia in streptozotocin-treated rats (Calcutt and Chaplan, 1997). Interestingly, the antinociceptive effects of intrathecal morphine were not observed in a nerve injury model of neuropathic pain, suggesting the possibility that tactile allodynia may be mediated by different physiological substrates in these different models of neuropathic pain (Calcutt and Chaplan, 1997).

The present study also demonstrated that the adenosine kinase inhibitor, 5′d-5IT had a significant antinociceptive effect on tactile allodynia in streptozotocin-treated diabetic rats. Pretreatment with the non-selective adenosine receptor antagonist theophylline significantly reduced the antinociceptive effects of 5′d-5IT. This observation is consistent with the potentiation of endogenous adenosine to activate its receptors as a mechanism of action underlying the analgesic effects of 5′d-5IT in this model. The current findings extend previous reports indicating that direct-acting adenosine receptor agonists have anti-allodynic effects in another model of neuropathic pain (L5/L6 spinal nerve ligation in rats). The pharmacology of the anti-allodynic properties of adenosine receptor modulation in that study was consistent with a role of the adenosine A_1 receptor subtype (Lee and Yaksh, 1996).

In addition to the demonstrated antinociceptive effects on tactile allodynia in the current study, adenosine kinase inhibitors have also been shown to reduce acute somatic nociception (Keil and DeLander, 1992; Kowaluk et al., 1998a), reduce flinching in the rat formalin model of persistent pain (Poon and Sawynok, 1995), and reduce carrageenan-induced thermal hyperalgesia (Kowaluk et al., 1998b; Poon and Sawynok, 1998), suggesting that this class of compounds may be useful analgesics in a broad spectrum of pain states. Adenosine receptor agonists, which have been studied more extensively than have adenosine kinase inhibitors, also have been shown to be efficacious in animal models of acute (Post, 1984; Karlsten et al., 1991) and neuropathic pain (Sosnowski and Yaksh, 1989; Yamamoto and Yaksh, 1992; Lee and Yaksh, 1996), and adenosine receptor agonists have demonstrated efficacy in human pain including neuropathies (Segerdahl et al., 1995; Sollevi et al., 1995). However, clinical use of direct acting adenosine receptor agonists may be limited by peripheral side effects such as sedation, bradycardia, and hypotension (Williams and Burnstock, 1997). Given that adenosine kinase inhibitors have been shown to selectively increase extracellular adenosine levels at neuronal sites of hyperexcitability or injury (Britton et al., 1996), inhibition of adenosine kinase may enhance the endogenous antinociceptive actions of adenosine while minimizing the potential for non-specific side effects. The present findings indicate that adenosine kinase inhibitors may have therapeutic potential as novel analgesic agents in the treatment of neuropathic pain.

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