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Adenosine kinase inhibitors attenuate opiate withdrawal via adenosine receptor activation

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Abstract

Previous studies have demonstrated a role for adenosine in mediating opiate effects. This study examines the effects of indirect activation of adenosine receptors, via treatment with adenosine kinase inhibitors, on the expression of opiate withdrawal in mice. Mice receive chronic morphine treatment via implantation of subcutaneous morphine pellets (75 mg) for 72 h. Mice then receive parenteral treatment with adenosine kinase inhibitors, either 5′-amino-5′-deoxyadenosine (2, 5, 20, 40 mg/kg, intraperitoneal or i.p.) or iodotubericidin (1, 2, 5 mg/kg, i.p.), followed by naloxone injection and opiate withdrawal signs are measured over 20 min. Both adenosine kinase inhibitors significantly reduce the following opiate withdrawal signs in a dose-dependent manner compared to vehicle: withdrawal jumps, teeth chattering, forepaw tremors, and forepaw treads. Additionally, 5′-amino-5′-deoxyadenosine significantly reduces withdrawal-induced diarrhea and weight loss. Effects of 5′-amino-5′-deoxyadenosine (40 mg/kg) on opiate withdrawal signs appear to be mediated via adenosine receptor activation as they are reversed by pretreatment by adenosine receptor antagonist caffeine (20 mg, i.p.) but not by selective phosphodiesterase inhibitor Ro 20-1724 (10 mg/kg, i.p.). Adenosine receptor activation via adenosine kinase inhibitor treatment attenuates opiate withdrawal and these agents may be generally useful in the treatment of drug withdrawal syndromes.

Keywords: Morphine; Opiate dependence; Opiate withdrawal; Adenosine; Purinergic receptor; (Mouse)

1. Introduction

Since opiate dependence causes enormous medical, psychological, social, occupational and legal difficulties, it is essential to develop a more complete understanding of its etiology and also potential new treatments. Chronic treatment with opiates results in behavioral consequences such as physical dependence and withdrawal syndromes. The mechanisms by which opiates produce these behavioral effects are incompletely understood, however changes in dopaminergic (Acquas and DiChiara, 1992; Diana et al., 1995), purinergic (Kaplan et al., 1994; Kaplan and Sears, 1996; Kaplan and Leite-Morris, 1997; Salem and Hope, 1997) and cAMP signaling (Terwilliger et al., 1991; Nestler, 1997; Kaplan et al., 1998) all appear relevant.

Many studies have demonstrated a role for the neuromodulator adenosine and adenosine receptors in mediating opiate effects. Extracellular adenosine is produced via neuronal metabolism of the neurotransmitter adenosine triphosphate (ATP) by membrane-bound ectonucleotidases and by cytosolic 5′-nucleotidases, or by hydrolysis via *S*-adenosylhomocysteine. Extracellular adenosine levels are regulated by a bi-directional nucleoside transporter. Once taken up by neuronal cells, adenosine is either phosphorylated by adenosine kinase or deaminated by adenosine deaminase (Meghji, 1991). Extracellular adenosine acts at pre- and post-synaptic receptor sites. Adenosine A_1 receptors have been localized to multiple brain regions involved in drug addiction including the nucleus accumbens, ventral tegmental area, substantia nigra, striatum, and frontal cortex (Rivkees et al., 1995). Adenosine A_{2A} receptors are localized to the caudate/putamen, nucleus accumbens, globus pallidus and olfactory tubercle (Johansson and Fredholm, 1995). Adenosine receptor sites, A_1 and A_{2A}, are negatively and positively coupled to the adenylyl cyclase, respectively. Because of their localization, important neurotransmitter interactions, and role in regulation of

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intracellular cAMP levels, adenosine receptors could alter mechanisms of opiate dependence.

A role for adenosine and adenosine receptors in mediating opiate dependence is supported by a variety of findings. Treatment with adenosine receptor agonists inhibited the development of tolerance to opiate antinociception (Contreras et al., 1990; Germany et al., 1990). Adenosine receptor agonist treatment inhibited the expression of opiate withdrawal (Germany et al., 1990; Dionyssopoulos et al., 1992; Kaplan and Sears, 1996; Salem and Hope, 1997) while adenosine receptor antagonist treatment enhanced withdrawal signs (Kaplan and Sears, 1996; Salem and Hope, 1997). Several studies support the hypothesis that chronic opiate effects are mediated by increases in purinergic signaling. Receptor binding studies have shown that increases in both adenosine A_1 receptors (Ahlijanian and Takemori, 1986; Kaplan et al., 1994) and in adenosine transporter sites (Kaplan and Leite-Morris, 1997) develop in brain tissues of opiate dependent mice. Additionally, chronic opiate treatment produced increases in adenosine A_1 receptor-mediated inhibition of γ-aminobutyric acid-(GABA) transmission in the ventral tegmental area, possibly resulting in mesolimbic dopaminergic activation (Bonci and Williams, 1996).

An indirect way to activate adenosine receptor-mediated signaling is via treatment with adenosine kinase inhibitors. Two adenosine kinase inhibitors, 5′-amino-5′-deoxyadenosine and iodotubericidin, enhanced extracellular adenosine release in spinal cord slices (Golembiowska et al., 1996) and increased glutamate-stimulated adenosine release in cortex (White, 1996). Similarly, iodotubericidin significantly increased adenosine release in hippocampal slices (Lloyd and Fredholm, 1995). In addition to inhibiting adenosine kinase function, iodotubericidin blocked nucleoside transport function in cells (Parkinson and Geiger, 1996). By inhibiting adenosine metabolism and enhancing adenosine release, adenosine kinase inhibitors increase extracellular adenosine levels and this results in increased activation of adenosine receptors.

Enhancement of extracellular adenosine levels by adenosine kinase inhibitors has produced significant in vivo effects. Spinal administration of adenosine kinase inhibitor 5′-amino-5′-deoxyadenosine produced antinociception (Keil and Delander, 1994; Poon and Sawynok, 1995) and increased opiate antinociception (Keil and Delander, 1994). Administration of both 5′-amino-5′-deoxyadenosine and iodotubericidin to rat cortex produced dose-dependent protection against chemoconvulsant-induced seizures (Zhang et al., 1993). Several studies have shown that peripheral administration of adenosine kinase inhibitors produces relevant central effects. Intraperitoneal administration of adenosine kinase inhibitors increased striatal adenosine concentrations, in the presence of kainic acid (Britton et al., 1996). Treatment (i.p.) with adenosine kinase inhibitor protected rats against transient focal ischemia and reduced behavioral signs of neurological deficits (Jiang et al., 1997). Intraperitoneal treatment with iodotubericidin and 5′-amino-5′-deoxyadenosine blocked seizures in mice (Zimring et al., 1995) and produced antinociceptive effects in mice (Kowaluk et al., 1996).

Given our understanding of the role of adenosine in mediating opiate effects and of the central effects of parenterally administered adenosine kinase inhibitors, we examined the effects of adenosine kinase inhibitors (i.p. treatment) on opiate withdrawal. Based on the above cited studies, 5′-amino-5′-deoxyadenosine and iodotubericidin are chosen as appropriate agents for parenteral administration. Doses and timing of drug administration used in this current study are based on data from these previous studies and from pilot data. To determine if adenosine kinase inhibitor effects are mediated by adenosine receptor activation, the reversibility of effects are investigated via pretreatment with adenosine receptor antagonist, caffeine. We examine if caffeine-induced reversibility of adenosine kinase inhibitor is mimicked by a selective phosphodiesterase inhibitor, Ro 20-1724, to ascertain if caffeine's effects are due to adenosine antagonism or to phosphodiesterase inhibition.

2. Methods

2.1. Animals

Male CD-1 mice (7–12 weeks old) from Charles River Laboratories (Wilmington, MA) are housed in a temperature and humidity-controlled environment on a 12-h light/dark cycle. Animals are given laboratory chow and water ad libitum. Mice are allowed to acclimate to the new environment at least 5 days before they are used in experiments.

2.2. Procedure

Mice are implanted with morphine pellets within the dorsal subcutaneous tissues after being anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneal or i.p.). Morphine pellets are left in place for 72 h. After 72 h of morphine treatment, adenosine kinase inhibitors or vehicle solution are administered i.p. to mice and 20 min later an opiate withdrawal syndrome is induced using an opiate receptor antagonist, naloxone HCl (5 mg/kg, i.p.) and withdrawal behaviors are measured in subjects over a 20 min period as described below.

In a separate study, caffeine (20 mg/kg, i.p.), or i.p. saline, is given to morphine-treated mice (72 h pellet implantation) and 20 min later adenosine kinase inhibitor, 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.), or 15% polyethylene glycol vehicle, is administered. Twenty minutes later, an opiate withdrawal syndrome is induced using naloxone and withdrawal behaviors are quantitated. In another study, selective phosphodiesterase inhibitor Ro

20-1724 (20 mg/kg, i.p.), or i.p. saline, is given to morphine-treated mice and 20 min later adenosine kinase inhibitor 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.), or 15% polyethylene glycol vehicle, is administered. Opiate withdrawal is induced using naloxone 20 min later as above.

Immediately after naloxone injection, animals are individually placed into a Plexiglas cylinder (15 cm diameter and 61 cm high) and are videotaped. The withdrawal behavioral assessment is based on the procedures of Kaplan and Sears (1996). The following behaviors are quantitated in individual subjects over a 20 min period: number of jumps, number of wet dog shakes, percent weight loss (percentage of pre-withdrawal value), and amount of diarrhea (in gram amounts from filter paper at base of cylinder). The following signs are checked as present (score of 1) or absent (score of 0) for each 1 min interval (maximum possible score is 20): teeth chattering, forepaw treading, and forepaw tremor.

2.3. Drugs

Morphine (75 mg) pellets is obtained from the National Institute on Drug Abuse-Research Technology Branch (Rockville, MD). Iodotubericidin, or 4-amino-5-iodo-7[*b*-D-ribofuranosyl]pyrrolo[2,3-*d*]-pyrimidine, and Ro 20-1724, or 4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone, are obtained from Research Biochemical International (Natick, MA). Naloxone, caffeine and 5′-amino-5′-deoxyadenosine is obtained from Sigma (St. Louis, MO). Adenosine kinase inhibitors and Ro 20-1724 are dissolved in 15% polyethylene glycol solution, naloxone is dissolved in sterile water and caffeine is dissolved in sterile 0.9% sodium chloride solution. All purinergic drugs are administered in a volume of 0.22 ml while naloxone is given in a volume of 0.15 ml.

2.4. Statistical analyses

Analysis of variance (ANOVA) procedures is used to test the significance of differences in withdrawal sign values at the different drug doses. If a significant ($P < 0.05$) P value is achieved for a given withdrawal sign (unless otherwise indicated), then post-hoc comparisons between groups are made using a Student–Newman–Keuls test.

3. Results

We have previously demonstrated that morphine pellet-implanted mice, as compared to vehicle pellet-implanted mice, showed significant increases in opiate withdrawal signs of jumping, wet-dog shakes, forepaw treads, forepaw tremors and diarrhea after naloxone injection (Kaplan et al., 1994). These effects are similar to the opiate withdrawal syndrome found in other rodent models (Contreras et al., 1990; Germany et al., 1990; Salem and Hope, 1997) and suggest the development of opiate dependence in this morphine treatment paradigm in mice.

Fig. 1 illustrates that pretreatment with both adenosine kinase inhibitors, 5′-amino-5′-deoxyadenosine and iodotu-

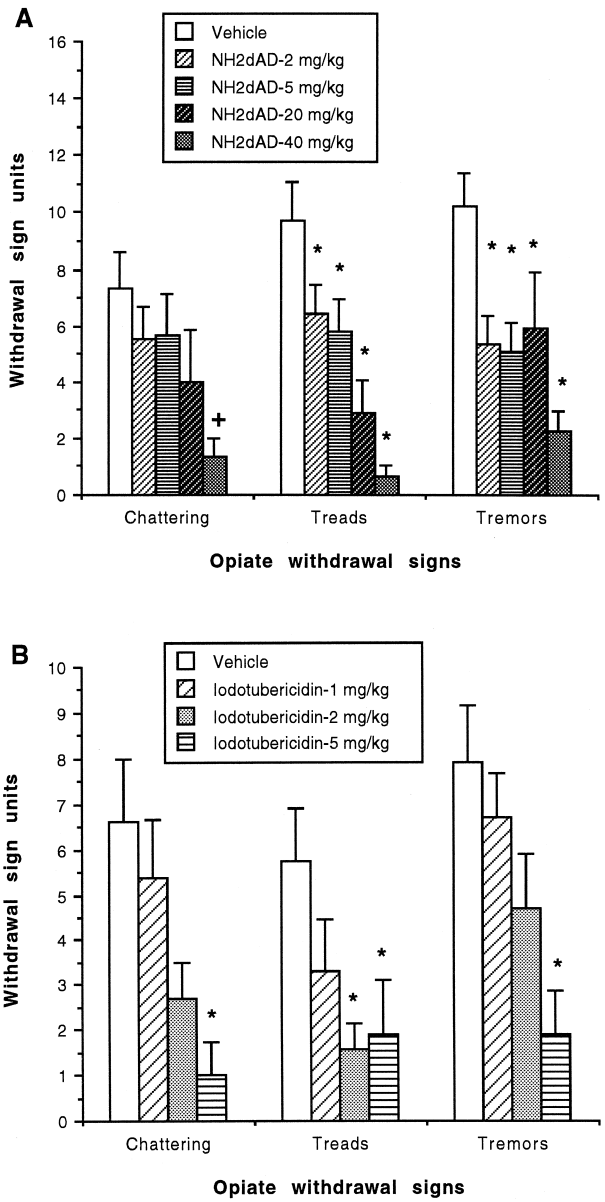

Fig. 1. Effects of treatment with adenosine kinase inhibitors 5′-amino-5′-deoxyadenosine (A) and iodotubericidin (B) on 'scored' naloxone-precipitated opiate withdrawal signs. Mice are made opiate dependent using morphine pellet implantation over a 72 h period. They are then pretreated with purinergic agents 20 min prior to the induction of opiate withdrawal (using naloxone 5 mg/kg, i.p.). Withdrawal signs are scored for 20 min. Each bar represents the mean value (±S.E.M.) for the withdrawal sign for 8–16 mice. $*$ $P < 0.05$ versus vehicle control treatment using post-hoc Student–Newman–Keuls test after ANOVA demonstration of significant differences between all treatment groups. $^{+}$ $P < 0.05$ versus vehicle control treatment using post-hoc Student–Newman–Keuls test after ANOVA demonstration of differences ($P = 0.08$) between groups.

bericidin, attenuated 'scored' opiate withdrawal signs of teeth chattering, forepaw treads and tremors. ANOVA procedures show that 5′-amino-5′-deoxyadenosine produces significant reductions in forepaw treads ($F[4,52] = 8.9$; $P < 0.0001$), and forepaw tremors ($F[4,52] = 5.4$; $P < 0.001$), and reduces teeth chattering ($F[4,52] = 2.2$; $P = 0.08$). Student–Newman–Keuls post-hoc testing indicates that differences between treatment groups and vehicle controls are significant ($P < 0.05$) for teeth chattering at 40 mg/kg, for treads at 2, 5, 20 and 40 mg/kg and for tremors at 2, 5, 20, 40 mg/kg. ANOVA procedures show that iodotubericidin significantly attenuates teeth chattering ($F[3,47] = 4.1$; $P < 0.05$), forepaw treads ($F[3,47] = 3.6$; $P < 0.05$), and forepaw tremors ($F[3,47] = 4.2$; $P < 0.05$). Student–Newman–Keuls testing shows differences between treatment groups and vehicle controls are significant for teeth chattering and tremors at the dose of 5 mg/kg and are significant for treads at doses of 2 and 5 mg/kg.

Fig. 2 shows that pretreatment with 5′-amino-5′-deoxyadenosine reduces 'counted' (or measured) withdrawal signs including jumps, percent weight loss and diarrhea; iodotubericidin attenuates withdrawal jumps only. ANOVA procedures show that 5′-amino-5′-deoxyadenosine reductions in jumps ($F[4,52] = 2.8$; $P < 0.05$), percent weight loss ($F[4,52] = 2.6$; $P < 0.05$), and diarrhea ($F[4,52] = 7.6$; $P = 0.0001$) are significant. Student–Newman–Keuls testing indicates that differences between treatment groups and vehicle controls are significant for jumps at 40 mg/kg, for diarrhea 2, 5, 20, 40 mg/kg and there are no individual dosage differences (vs. vehicle) for weight loss. ANOVA demonstrates that iodotubericidin significantly inhibits jumps ($F[3,47] = 4.8$; $P < 0.01$) and the Student–Newman–Keuls test shows that differences are significant between vehicle and 5 mg/kg groups.

Fig. 3 illustrates the effects of caffeine pretreatment on adenosine kinase inhibitor-induced attenuation of opiate withdrawal signs. Caffeine is used to reverse adenosine kinase effects because of its known effects as an adenosine receptor antagonist (Daly, 1993). Each subject receives pretreatment with caffeine (20 mg/kg) or saline for 20 min followed by treatment with 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.) or 15% polyethylene glycol vehicle for 20 min and then undergoes naloxone-induced withdrawal. The figure shows that adenosine kinase inhibitor treatment reduces opiate withdrawal signs of forepaw treads, tremors, teeth chattering and jumps and caffeine pretreatment blocks this attenuation. ANOVA procedures show significant differences between combination treatments for measures of forepaw treads ($F[2,32] = 5.9$; $P < 0.01$), tremors ($F[2,32] = 15.7$; $P < 0.0001$) and teeth chattering ($F[2,32] = 6.9$; $P < 0.005$) and there are modest differences for withdrawal diarrhea ($F[2,32] = 2.1$; $P = 0.13$). For forepaw treads and tremors, Student–Newman–Keuls testing indicates significant differences ($P < 0.05$) between the saline/5′-amino-5′-deoxyadenosine group and both saline/vehicle and caffeine/5′-amino-5′-deoxyadenosine groups. For teeth chattering, Student–Newman–Keuls testing indicates significant differences between the saline/vehicle group and both saline/5′-amino-5′-deoxyadenosine and caffeine/5′-amino-5′-deoxyadenosine groups. The caffeine/5′-amino-5′-deoxyadenosine group values are higher than those of the saline/5′-amino-5′-deoxyadenosine group and are similar to values from the saline/vehicle group. For withdrawal jumps, ANOVA demonstrates significant differences ($F[2,32] = 4.5$; $P < 0.05$) between the three treatment groups. For this measure, Student–Newman–Keuls testing shows significant differences ($P < 0.05$) between saline/5′-amino-5′-

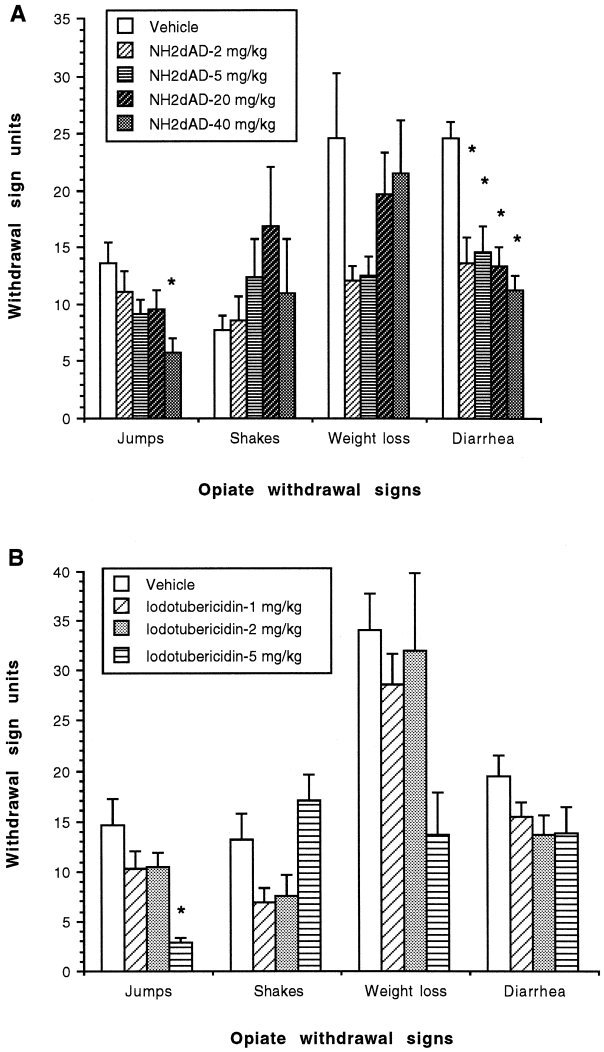

Fig. 2. Effects of treatment with adenosine kinase inhibitors 5′-amino-5′-deoxyadenosine (A) and iodotubericidin (B) on 'counted' naloxone-precipitated opiate withdrawal signs. Morphine dependent mice are pretreated with adenosinergic agents 20 min prior to the induction of opiate withdrawal (using naloxone 5 mg/kg, i.p.). Each bar represents the mean value (±S.E.M.) for the withdrawal sign for 8–16 mice. Withdrawal jumps are divided by 10, percent weight loss is multiplied by 10 and diarrhea (g) is multiplied by 100 so that all measures can be plotted on this same graph. $*$ $P < 0.05$ versus vehicle control treatment using Student–Newman–Keuls test after ANOVA demonstration of significant differences between all treatment groups.

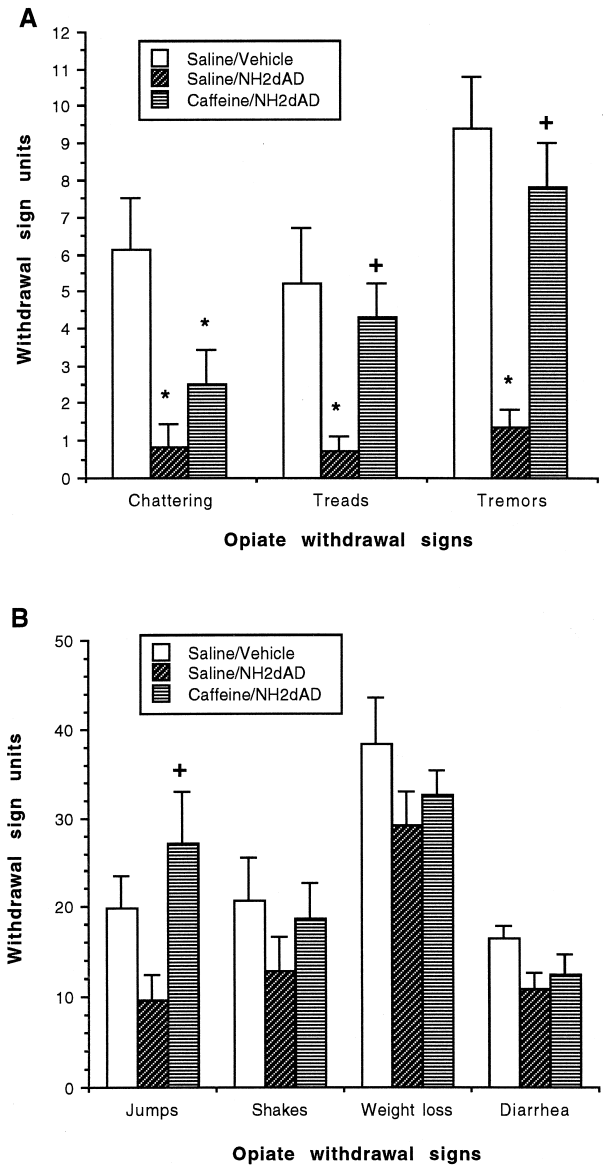

Fig. 3. Effects of pretreatments of caffeine (20 mg/kg, i.p.) and 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.) on scored (A) or counted (B) naloxone-precipitated opiate withdrawal signs. Morphine dependent mice are pretreated with caffeine (for 20 min) followed by 5′-amino-5′-deoxyadenosine (for 20 min) followed by naloxone (5 mg/kg, i.p.) induction of opiate withdrawal. Each bar represents the mean value (±S.E.M.) for the withdrawal sign for 11–12 mice. Withdrawal jumps are divided by 10, percent weight loss is multiplied by 10 and diarrhea (g) is multiplied by 100. $*\ P < 0.05$ versus saline/vehicle control treatment using post-hoc Student–Newman–Keuls test after ANOVA demonstration of significant differences between all treatment groups; $^{+}P < 0.05$ versus saline/5′-amino-5′-deoxyadenosine treatment.

deoxyadenosine and caffeine/5′-amino-5′-deoxyadenosine groups. In summary, adenosine kinase inhibitor reduces opiate withdrawal-induced treads, tremors, teeth chattering, and jumps and caffeine pretreatment blocks these effects.

Fig. 4 illustrates the effects of pretreatment with selective phosphodiesterase inhibitor, Ro 20-1724, on adenosine kinase inhibitor-induced attenuation of opiate withdrawal signs. This treatment is used to ascertain if caffeine effects are due to adenosine receptor antagonism or phosphodiesterase inhibition (Daly, 1993). Each subject receives Ro 20-1724 (10 mg/kg) or saline for 20 min followed by 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.) or 15% polyethylene glycol vehicle for 20 min and then undergoes

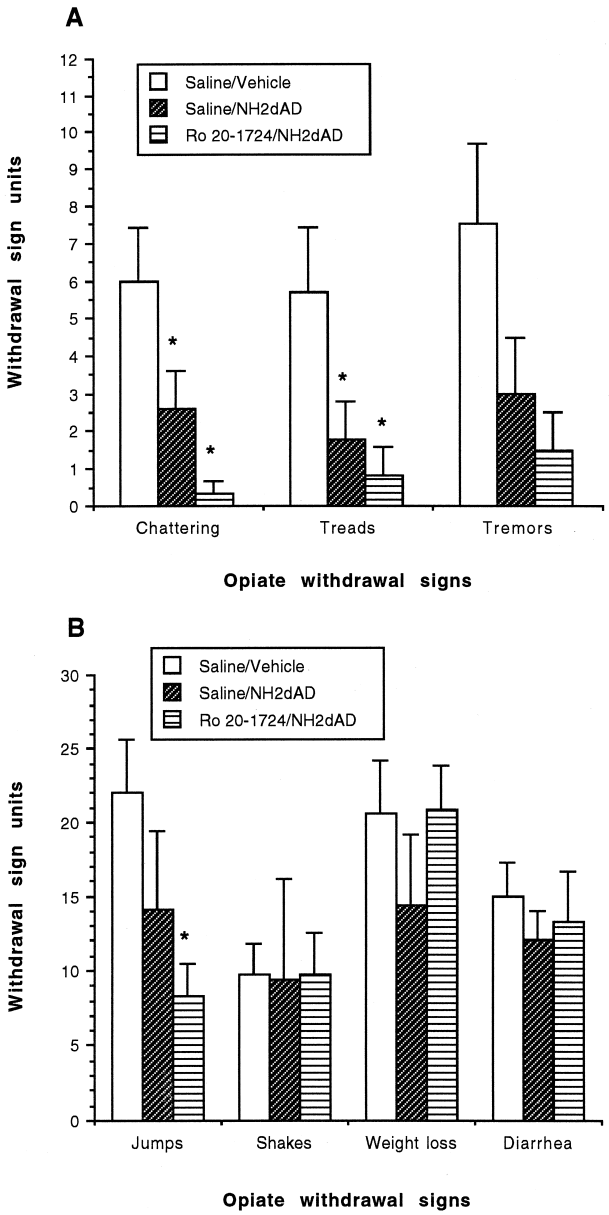

Fig. 4. Effects of pretreatments of Ro 20-1724 (3 mg/kg, i.p.) and 5′-amino-5′-deoxyadenosine (40 mg/kg, i.p.) on scored (A) or counted (B) naloxone-precipitated opiate withdrawal signs. Morphine dependent mice are pretreated with Ro 20-1724 (for 20 min) followed by 5′-amino-5′-deoxyadenosine (for 20 min) followed by naloxone (5 mg/kg, i.p.) induction of withdrawal. Each bar represents the mean value (±S.E.M.) for the withdrawal sign for 5–6 mice. Withdrawal jumps are divided by 10 and percent weight loss is multiplied by 10, and diarrhea (g) is multiplied by 100. $*\ P < 0.05$ versus saline/vehicle control treatment using post-hoc Student–Newman–Keuls test after ANOVA demonstration of significant differences between treatment groups.

naloxone-induced withdrawal. This dose of Ro 20-1724 is chosen based on its demonstration of behavioral effects in an another mouse model (Imaizumi et al., 1994). ANOVA procedures show significant differences between treatments for measures of forepaw treads ($F[2,14] = 4.3$; $P < 0.05$), tremors ($F_{2,14} = 3.6$; $P = 0.05$) and teeth chattering ($F[2,14] = 8.1$; $P < 0.005$) and jumps ($F[2,14] = 3.7$; $P < 0.05$). For teeth chattering and treads, Student–Newman–Keuls testing indicates significant differences between the saline/vehicle group and both saline/5′-amino-5′-deoxyadenosine and Ro 20-1724/5′-amino-5′-deoxyadenosine groups. For forepaw tremors, there were no individual group differences between treatments. For jumps, Student–Newman–Keuls testing shows significant differences between saline/vehicle and Ro 20-1724/5′-amino-5′-deoxyadenosine groups. In all of the above cases, Ro 20-1724/5′-amino-5′-deoxyadenosine group values are lower than those from the other two groups. Unlike caffeine, Ro 20-1724 does not reverse adenosine kinase inhibitor-induced attenuation of opiate withdrawal signs (teeth chattering, treads, tremors and jumps) and instead reduces withdrawal.

4. Discussion

There are a number of studies which have demonstrated a functional role for adenosine receptors in mediating acute and chronic opiate effects. In this study, two adenosine kinase inhibitors significantly attenuate opiate withdrawal signs. Both 5′-amino-5′-deoxyadenosine and iodotubericidin significantly reduce the following opiate withdrawal signs compared to vehicle: teeth chattering, forepaw tremors, forepaw treads, and withdrawal jumps. Additionally, 5′-amino-5′-deoxyadenosine significantly reduces percent weight loss and diarrhea. These results are strengthened by findings that drug effects are dose-dependent. The findings are consistent for the types of withdrawal behaviors affected as both drugs reduce jumps, teeth chattering, tremors and treads. The observation that adenosine kinase inhibitors reduce opiate withdrawal signs is consistent with findings of other purinergic drugs suppressing opiate withdrawal (Germany et al., 1990; Dionyssopoulos et al., 1992; Kaplan and Sears, 1996; Salem and Hope, 1997). Our results are consistent with other studies which have demonstrated central effects from parenteral administration of adenosine kinase inhibitors, including anticonvulsant (Zimring et al., 1995), antinociceptive (Kowaluk et al., 1996) and neuroprotective (Jiang et al., 1997) effects. However, peripheral effects of purinergic drugs, including hypotension, bradycardia, and respiratory stimulation could also modify the expression of withdrawal signs. Future studies using intracerebral administration of these drugs can fully determine if the opiate withdrawal effects demonstrated here result from central mechanisms.

We hypothesize that adenosine kinase inhibitor treatment increases endogenous adenosine levels and increased activation of adenosine receptors. Other studies have confirmed this effect by adenosine kinase inhibitors in vitro in cortex (White, 1996) and in hippocampus (Lloyd and Fredholm, 1995) and in vivo in striatum (Britton et al., 1996). Pretreatment with non-selective adenosine receptor antagonist caffeine blocks the attenuation of opiate withdrawal signs by adenosine kinase inhibitor. Specifically, 5′-amino-5′-deoxyadenosine treatment reduces withdrawal jumps, treads, tremors, and teeth chattering and caffeine pretreatment blocks this attenuation. Since caffeine produces phosphodiesterase inhibition, we examine if its blockade of adenosine kinase inhibitor effects relate to this mechanism. Potent and selective cAMP phosphodiesterase inhibitor, Ro 20-1724, does not reverse adenosine kinase inhibitor-induced attenuation of withdrawal. These findings support the premise that caffeine effects are due to adenosine receptor antagonism and that adenosine kinase inhibitor effects are mediated by adenosine receptor activation. Since caffeine is equipotent as an antagonist at adenosine A_1 and A_{2A} receptors (Daly, 1993), one cannot conclude which receptor subtype is relevant to opiate withdrawal.

There are several potential mechanisms which could explain the effects of adenosine kinase inhibitors and phosphodiesterase inhibitor treatments on opiate withdrawal. Each mechanism involves the activation of specific adenosine receptors in different brain regions and follow-up studies must ascertain which mechanisms are most relevant. Adenosine receptor activation of neurons projecting from the ventral tegmental area (or A10 neurons) has been shown to alter mesolimbic dopaminergic neuronal cell firing and potentially affect opiate withdrawal. Chronic opiate treatment followed by abrupt discontinuation produces a withdrawal syndrome which is associated with profound reductions in both mesolimbic dopaminergic neuronal cell firing (Diana et al., 1995) and dopamine release by these neurons (Acquas and DiChiara, 1992). Adenosine A_1 receptors are found presynaptically on GABAergic cells in the VTA (Wu et al., 1995; Bonci and Williams, 1996) and appear to inhibit GABA release. Peripheral administration of adenosine A_1 receptor agonist activates A10 neurons and increases dopamine efflux in the nucleus accumbens (Murai et al., 1994). Conversely, peripheral administration of caffeine reduces firing rates of A10 neurons in a dose-dependent fashion (Stoner et al., 1988).

Treatment with adenosine kinase inhibitors could activate adenosine A_1 receptors on mesolimbic dopaminergic neurons and oppose reductions in dopaminergic transmission which mediate withdrawal. Furthermore, prolonged increases in intracellular cAMP levels can produce enhanced cAMP transport to extracellular sites and metabolism to adenosine (Brundege et al., 1997), resulting in enhanced activation of midbrain adenosine A_1 receptors, a possible compensatory mechanism of adaptation

(Bonci and Williams, 1996). Potent and selective cAMP phosphodiesterase inhibitor, Ro 20-1724, also appears to attenuate opiate withdrawal (Fig. 4). Ro 20-1724 treatment could produce prolonged increases in intracellular cAMP levels, enhanced metabolism to adenosine, and activation of adenosine A_1 receptors (Brundege et al., 1997). Since adenosine tonically stimulates midbrain dopamine neurons via the adenosine A_1 receptor activation (Murai et al., 1994), increases in adenosine levels could stimulate mesolimbic dopamine systems and reduce opiate withdrawal (Diana et al., 1995).

Another possible mechanism for purinergic drugs in opiate withdrawal relates to their effects on adenylyl cyclase activity in brain regions including the locus coeruleus and striatum. Chronic opiate treatment and opiate withdrawal produces adaptive upregulation of adenylyl cyclase activity in dorsal (Kaplan et al., 1998) and ventral striatum (Terwilliger et al., 1991) and in locus coeruleus (Terwilliger et al., 1991). One hypothesis is that upregulated cAMP pathways in ventral striatum contributes to the aversive state associated with withdrawal while upregulation of cAMP in locus coeruleus produces the somatic signs of opiate withdrawal (Nestler, 1997). Locus coeruleus, dorsal striatum and nucleus accumbens are regions which contain adenosine A_1 receptors (Regenold and Illes, 1990; Rivkees et al., 1995) that are negatively coupled to adenylyl cyclase. Adenosine A_1 activation in these regions by adenosine kinase inhibitor treatment could oppose upregulated cAMP systems and attenuate opiate withdrawal. This does not appear to be a relevant mechanism for Ro 20-1724 based on our findings.

Finally, adenosine kinase inhibitor treatment could block drug withdrawal syndromes via their anticonvulsant effects. Drug withdrawal signs reflects, in part, hyperexcitability of the central nervous system as demonstrated by signs of tremors, irritability, rigidity, and enhanced susceptibility to seizures (Watson and Little, 1995). Intracerebral administration of adenosine A_1 receptor agonists and adenosine kinase inhibitors to prepiriform cortex produces anticonvulsant effects (Zhang et al., 1993). Adenosine A_1 receptor agonists reduce drug withdrawal-induced tremors and seizures elicited in rats while pretreatment selective A_1 receptor antagonist completely abolished this effect (Concas et al., 1994). Adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyladenosine, inhibited ethanol-induced withdrawal tremors, seizures and deaths (Concas et al., 1994) while non-selective adenosine receptor agonist treatment reduced pentobarbital-induced withdrawal scores and convulsions (Germany and Contreras, 1994). Adenosine kinase inhibitors, as anticonvulsants, could similarly reduce drug withdrawal-induced central nervous system excitability.

Only partially effective drugs, such as clonidine, or addictive substances, such as methadone, are available for the treatment of opiate withdrawal. There is significant potential for the future use of purinergic drugs, such as adenosine kinase inhibitors, in the treatment of drug dependence. Purinergic drugs have been used for the treatment of other neuropsychiatric purposes such as cognitive enhancement, analgesia, neuroprotection, anxiolysis and for anticonvulsant effects (Snyder, 1997). The advantage of adenosine kinase inhibitors over adenosine receptor agonists may relate to their improved side effect profile (Zimring et al., 1995) and further pre-clinical trials are warranted.

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