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ORIGINAL INVESTIGATION

The effects of adenosine A_{2A} receptor antagonists on haloperidol-induced movement disorders in primates

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Abstract
Rationale Adenosine and dopamine interact within the striatum to control striatopallidal output and globus pallidus GABA release. Manipulating striatal adenosine transmission via blockade of the A_{2A} receptor subtype can compensate for the reduced dopamine activity within the striatum that underlies movement disorders such as antipsychotic-induced extrapyramidal syndrome (EPS) and Parkinson's disease (PD). Preclinical studies in the rat have demonstrated that adenosine A_{2A} receptor antagonists can attenuate behaviors reflecting reduced dopamine activity, such as haloperidol-induced catalepsy and hypoactivity.
Objectives In the present studies using nonhuman primates, adenosine antagonists were tested against haloperidol-induced EPS in *Cebus apella* and haloperidol-induced catalepsy in *Saimiri sciureus* (squirrel monkey). Specifically, the A_{2A} receptor antagonists, SCH 412348 (0.3–30 mg/kg PO) and KW-6002 (3–100 mg/kg PO); the A_1/A_{2A} receptor antagonist, caffeine (1–30 mg/kg PO and IM); and the A_1 receptor antagonist, DPCPX (3–30 mg/kg PO) were tested in at least one of these models.
Results SCH 412348 (10–30 mg/kg), KW-6002 (57–100 mg/kg), and caffeine (30 mg/kg) significantly increased the time to EPS onset. Additionally, SCH 412348, KW-6002, and caffeine afforded protection from the onset of EPS for at least 6 h in some of the primates. SCH 412348 (10 mg/kg) and caffeine (10 mg/kg) significantly reduced haloperidol-induced catalepsy. DPCPX produced a very slight attenuation of EPS at 30 mg/kg, but had no effect on catalepsy.
Conclusions These findings suggest that adenosine A_{2A} receptor antagonists may represent an effective treatment for the motor impairments associated with both antipsychotic-induced EPS and PD.

Keywords Nonhuman primate · Dopamine · Adenosine · A_{2A} receptor · Movement disorders · Parkinson's disease · Extrapyramidal syndrome · Antipsychotics · Schizophrenia

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Introduction

The purine, adenosine, is a ubiquitous modulator of neuronal function in the central and peripheral nervous systems that interacts with a number of major neurotransmitter systems, including the glutamatergic, cholinergic, GABAergic, and dopaminergic systems (Kurokawa et al. 1996; Latini et al. 1996; Mori and Shindou 2003; Popoli et al. 2003). Adenosine has been shown to exert its biological actions via the P1 class of G protein-coupled purinergic receptors. Based on their pharmacology, signal transduction mechanisms, and amino acid sequence homology, the P1 class of receptors is further subdivided into four receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3 (Fredholm et al. 2001; Jacobson and Gao 2006).

The A_{2A} receptor is of particular interest as it appears to play a prominent role in the established interaction of adenosine and dopamine (DA) within the striatum (Ferre et al. 1992, 1993). More specifically, A_{2A} receptors are highly concentrated in discrete brain nuclei of the basal ganglia that are associated with the dopaminergic nigrostriatal and mesolimbic neuronal pathways (e.g., caudate–putamen, globus pallidus, nucleus accumbens, and olfactory tubercles) (Rosin et al. 1998; Svenningsson et al. 1999). Within the striatum, A_{2A} receptors are predominantly localized to the GABAergic, striatopallidal, enkephalin-expressing output neurons where they are colocalized with DA D_2 receptors (Pollack et al. 1993; Schiffmann and Vanderhaeghen 1993; Johansson et al. 1997; Hettinger et al. 2001). These output neurons form part of the 'indirect' pathway, which along with the 'direct' pathway projects to the globus pallidus and substantia nigra and act in an opposing manner to control movement (Alexander and Crutcher 1990; Gerfen 1992; Schiffmann et al. 2007). Activation of striatal A_{2A} and D_2 receptors leads to opposing actions with A_{2A} agonists inhibiting, and D_2 agonists increasing, GABA release in the globus pallidus. Conversely, inhibition of A_{2A} receptors facilitates, and inhibition of D_2 receptors reduces, GABA release (Mayfield et al. 1996; Ferre et al. 1997; Schiffmann et al. 2007). However, more recent evidence suggests that the processes by which the striatum controls output to areas of the brain are much more complex and involve a number of additional pathways (see Parent and Cicchetti 1998). Clearly, further studies are needed to clarify the role of the adenosine system and particularly the A_{2A} receptor (e.g., the importance of heteromer formation with A_1, D_2, CB1, and mGluR5 receptors) in modulating these complex processes and interactions (for review, see Schiffmann et al. 2007). However, there is clearly an intricate role of the A_{2A} receptor within the basal ganglia circuitry in the modulation of striatal function.

A number of movement disorders are clearly associated with impairments in dopaminergic neurotransmission and the resulting dysfunction in the 'direct' and 'indirect' output pathways of the striatum. These include Parkinson's disease (PD), which results from the degeneration of the nigrostriatal DA pathway, and the extrapyramidal syndrome (EPS), which is associated with the nonselective blockade of DA receptors within the striatum by antipsychotic drugs. Attempts to improve dopaminergic transmission in PD patients by treatment with the DA precursor, L-Dopa, or treatment with DA agonists have had limited success, primarily due to drug-induced side effects such as dyskinesia, somnolence, and compulsive behavior (Obeso et al. 1989; Marsden 1994; Cantor and Stern 2002; Driver-Dunckley et al. 2003). Adenosine A_{2A} receptor antagonists may offer an alternative approach to the treatment of PD by facilitating intrastriatal GABA release and restoring the 'indirect' inhibitory output from the striatum to the globus pallidus, subthalamic nucleus (STN), and thalamus. A_{2A} receptor antagonists may also lack the side effects associated with chronic DA receptor stimulation (Ferre et al. 1997; Richardson et al. 1997; Morelli et al. 2007).

Indeed, nonselective A_{2A} antagonists, such as caffeine, and more selective A_{2A} receptor antagonists, such as KW-6002 (istradefylline), have been shown to be effective in rodent and primate models of PD or reduced dopaminergic activity (Kanda et al. 1994; Mandhane et al. 1997; Kanda et al. 1998; Grondin et al. 1999; Shiozaki et al. 1999; Hauber et al. 2001). A_{2A} antagonists also appear to synergize with L-Dopa in animal models of PD, allowing the use of lower doses of L-Dopa to achieve efficacy with a concomitant reduction in dyskinesias (Pinna et al. 2001). Furthermore, KW-6002 has recently been shown to reduce off-time in PD patients when administered with L-Dopa and to allow the use of lower doses of L-Dopa to achieve a similar level of symptom relief with an accompanying reduction in dyskinesias (Bara-Jimenez et al. 2003; Chase et al. 2003; Hauser et al. 2003; Jenner 2005).

Antipsychotic-induced EPS is characterized by akathisia (restlessness), dystonia (muscular spasms of the neck, eyes, tongue, or jaw), drug-induced parkinsonism (muscle stiffness, shuffling gait, drooling, tremor), akinesia (inability to initiate movement), and tardive dyskinesia (repetitive, involuntary, purposeless movements usually of the face and limbs) and becomes evident following repeated dosing with antipsychotic drugs, particularly drugs with antagonist properties at DA D_2 receptors. While some of these EPS symptoms can be attenuated by anticholinergic drugs, such agents also present with prominent side effects that affect patient compliance. Based on the biology of the A_{2A} receptor and studies demonstrating the successful treatment of PD symptoms with A_{2A} receptor antagonists, we hypothesized that adenosine A_{2A} receptor antagonists would alleviate antipsychotic-induced EPS. The principal aim of these studies was to test this hypothesis using primate models of antipsychotic-induced EPS and catalepsy. While we and other investigators have examined the effects of adenosine receptor antagonists on drug-induced EPS and catalepsy in rodents (Kanda et al. 1994; Kafka and Corbett 1996; Malec 1997; Correa et al. 2004; Neustadt et al. 2007; Pinna et al. 2007), there is no such work in primates. To this end, the A_{2A} receptor antagonists, SCH 412348 (A_{2A} Ki=0.6 nM, A_1 Ki≥960 nM; Neustadt et al. 2007) and KW-6002 (A_{2A} Ki=2.2 nM, A_1 Ki=150 nM; Shimada et al. 1997); the nonselective adenosine receptor antagonist, caffeine; and the A_1 receptor antagonist, DPCPX (Lohse et al. 1987) were tested in two established primate models; haloperidol-induced EPS in *Cebus apella* monkeys (Gunne and Barany 1976; Weiss et al. 1977) and

haloperidol-induced catalepsy model in squirrel monkeys (Rosenzweig-Lipson and Bergman 1994).

Materials and methods

All procedures involving animals were conducted in an AAALAC-accredited facility in conformity with the institutional guidelines and in compliance with the NIH 'Guide to the Care and Use of Laboratory Animals' and the Animal Welfare Act.

Cebus apella EPS studies

Subjects

Seven male *C. apella* monkeys previously sensitized to chronically administered haloperidol were used in this study (see Coffin et al. 1989). Briefly, *C. apella* monkeys were given 0.3 mg/kg of haloperidol orally in a banana once a week until abnormal EPS-like movements were established (usually following 12–14 weeks of treatment). The subjects weighed between 3 and 4 kg and were at least 15 years of age. The animals were individually housed and maintained on a 12-h light/dark cycle. Water was continuously available in the home cages. Animals were fed a high-protein monkey chow ad libitum after testing. The subjects were also fed fresh fruit and vegetables at least three times a week and treats (PRIMA-treats, peanuts, worms, etc.) several times a week. On no-test days, enrichment in the form of durable rubber toys and exposure to television was provided. All experiments were carried out in the home cage during the light cycle (0700–1900 hours).

Procedure

Each *C. apella* monkey was resensitized to the effects of chronic haloperidol treatment. The subjects were exposed to haloperidol (0.3 mg/kg, PO, in a banana) once or twice a week until each of the subjects displayed consistent and reliable levels of EPS upon acute administration of the same dose of haloperidol (usually following three or four treatments). Baseline levels of EPS behaviors were established for each monkey by scoring of stable EPS behaviors following several haloperidol administrations.

On test days, each monkey was dosed with haloperidol (0.3 mg/kg, PO, in a banana) either with or without an adenosine antagonist. Two blinded observers scored the animals' behavior before dosing and every 30 min up to 6 h after dosing. The observers' scores were combined and averaged. A within-subjects crossover design was used to study each drug. The subjects were tested a maximum of 2 days per week and 2–5 days separated each testing day to allow for drug washout. The effect of haloperidol to induce EPS was monitored in each monkey across the studies to ensure that the EPS response was not affected by any drug carry-over effect. No significant shifts in EPS response to haloperidol in terms of either onset or severity were noted. To prevent injury, the muscarinic antagonist, scopolamine, was administered once monkeys exhibited full EPS. Full EPS for a particular sensitized animal is defined as the collection of behaviors typically induced by haloperidol in that animal. This full EPS profile has been well-characterized over 15+ years of behavioral observation (Coffin et al. 1989, 1992), and these profiles remain consistent from one EPS episode to the next. Behavioral scores for sensitized animals range from 15 to 20 (see below). Based on previous experiences demonstrating that EPS will persist for the full 6 h test if left untreated, the final EPS score of monkeys rescued with scopolamine prior to completion of the test was extrapolated out for the full 6 h test.

Behavioral scoring

Nine abnormal movements were scored at each time point using a scale of 0–4 depending on the severity of the behavior. A score of 0 was assigned for no occurrence of the behavior, 1 for minimal, 2 for mild, 3 for moderate, and 4 for severe. The abnormal movements scored were (1) perioral movements, (2) severe biting, (3) tongue protrusion, (4) upper limb choreic movements (irregular, spasmodic, involuntary movements of the limbs), (5) upper limb athetoid movements (slow writhing involuntary movements characterized by flexion, extension, pronation, and supination of the fingers, hands, toes, and feet), (6) lower limb choreic movements, (7) lower limb athetoid movements, (8) trunk rocking/twisting/head pushing, and (9) perseverative circling. Additionally, two global judgment scores were also assigned, namely, severity of abnormal movements and incapacitation due to abnormal movements. Based on this, a total maximum score of 44 was possible (4 for severity×11 scores), although the average behavioral score ranged from 15 to 20 as most behaviors were not severe in nature and a number of behaviors are mutually exclusive. Levels of catalepsy and tremor were also noted before the onset of EPS in some animals, but these scores were not included in the overall abnormal movement score.

EPS data were handled and analyzed in three ways. First, the mean EPS scores for the monkeys were calculated at each 30 min time slot during the 6-h test and the mean EPS score 210 min after haloperidol (the time point when all vehicle-treated monkeys exhibit full EPS) was analyzed. Additionally, based on the observation that some monkeys

remained EPS-free for the full 6-h test, the EPS score for each individual monkey was plotted at the 6-h time point to illustrate this effect. Finally, as there also appeared to be drug effects that resulted in a delay to the onset of EPS, the mean time of EPS onset post haloperidol was analyzed. Due to nature of the data (scores) and the variability in response (i.e., data was not normally distributed), all data from the EPS studies were analyzed using the nonparametric Friedman test (GraphPad Instat, San Diego, CA, USA) followed by a Dunn post hoc test with a significance level of $p<0.05$. Median and interquartile ranges (IQR) for each drug are also presented within the text.

Drugs

Haloperidol (0.3 mg/kg) was administered orally in a hollowed out piece of banana with a small amount of Nutri-Cal nutrient gel. SCH 412348 ((7-[2-[4-difluorophenyl)-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine) (0.3, 1, 3, 10, and 30 mg/kg), KW-6002 ((*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dhydro-1H-purine-2,6-dione) (3, 10, 30, 57, and 100 mg/kg), or DPCPX (1,3-dipropyl-8-cyclopentylxanthine) (3, 10, and 30 mg/kg) were administered in conjunction with the haloperidol in the same piece of banana. Caffeine (10 and 30 mg/kg) was administered orally in apple juice (to avoid the bitter taste of caffeine) either 30 or 60 min after haloperidol administration (depending on the baseline onset of EPS for each subject). Caffeine was administered orally as the procedure to conduct an intramuscular (IM) injection in the *C. apella* colony is very stressful to the animals and detrimental to the EPS. Scopolamine (0.1 mg/kg) was administered IM to terminate EPS behaviors after required behavioral readings. At the end of each study day, benztropine (3 mg/kg) was dosed orally, using the same methods as for haloperidol, to prevent EPS behaviors from reoccurring overnight.

Squirrel monkey catalepsy studies

Subjects

Three male squirrel monkeys (*Saimiri sciureus*) were used in these studies. The subjects weighed between 0.8 and 1 kg and were at least 6 years of age. The animals were individually housed and maintained on a 12-h light/dark cycle. Water was continuously available in the home cages. Animals were fed a high-protein monkey chow ad libitum after testing. The subjects were also fed fresh fruit and vegetables at least three times a week and treats (PRIMA-treats, peanuts, worms, etc.) several times a week. Enrichment in the form of durable rubber toys, hammocks, and perches was provided as well.

Procedure

The subjects were tested a maximum of 2 days per week with a minimum of 2 days separating each testing day to allow for drug washout. On testing days, the subjects were tested one at a time. The test subject was first dosed with either an adenosine antagonist or vehicle and returned to its home cage. After an appropriate pretreatment time, the subject was brought into a quiet room and seated in a Plexiglas primate chair for a 15-min habituation period. The subject was then dosed with haloperidol (0.03 mg/kg, IM). A monitor was used to view the monkey's behavior during the testing session. Twenty minutes later, a 5-min behavioral observation was recorded with a video camera and a blinded observer recorded the total time each animal spent in a cataleptic state. Using a method similar to Rosenzweig-Lipson and Bergman (1994), catalepsy was defined as immobility with eyes open, usually accompanied by unusual postures, including rigid limb extensions and/or a twisted torso. The catalepsy induced in these procedures was sensitive to the anticataleptic effects of scopolamine (data not shown). A within-subjects crossover design was used to study each drug. The time spent cataleptic for each drug dose/response study was analyzed using a one-factor, repeated-measures ANOVA with an accepted significance level of $p<0.05$.

Drugs

SCH 412348 (10 and 30 mg/kg) and DPCPX (10 and 30 mg/kg) were administered orally by gavage in 0.4% methylcellulose in sterile 0.9% saline. SCH 412348 and DPCPX were administered 2 and 1 h prior to haloperidol, respectively. Caffeine (1, 3, and 10 mg/kg) or vehicle (0.9% saline) were administered IM 10 min prior to haloperidol (0.03 mg/kg, IM in 0.9% saline). Scopolamine (0.1 mg/kg, in saline) was administered IM at the end of the test session to terminate catalepsy behaviors. KW-6002 was not tested in the catalepsy model due to a lack of compound.

Results

Cebus apella EPS studies

SCH 412348, KW-6002, and caffeine reduced the mean EPS score during the 6-h test (Fig. 1). Additionally, the data in Fig. 1 suggest that there was a small effect of DPCPX to attenuate haloperidol-induced EPS, at least during the first 90 min following haloperidol administration.

Each drug was measured for efficacy 3.5 h post administration, i.e., the time taken for vehicle-treated controls to reach the full EPS profile following haloperidol

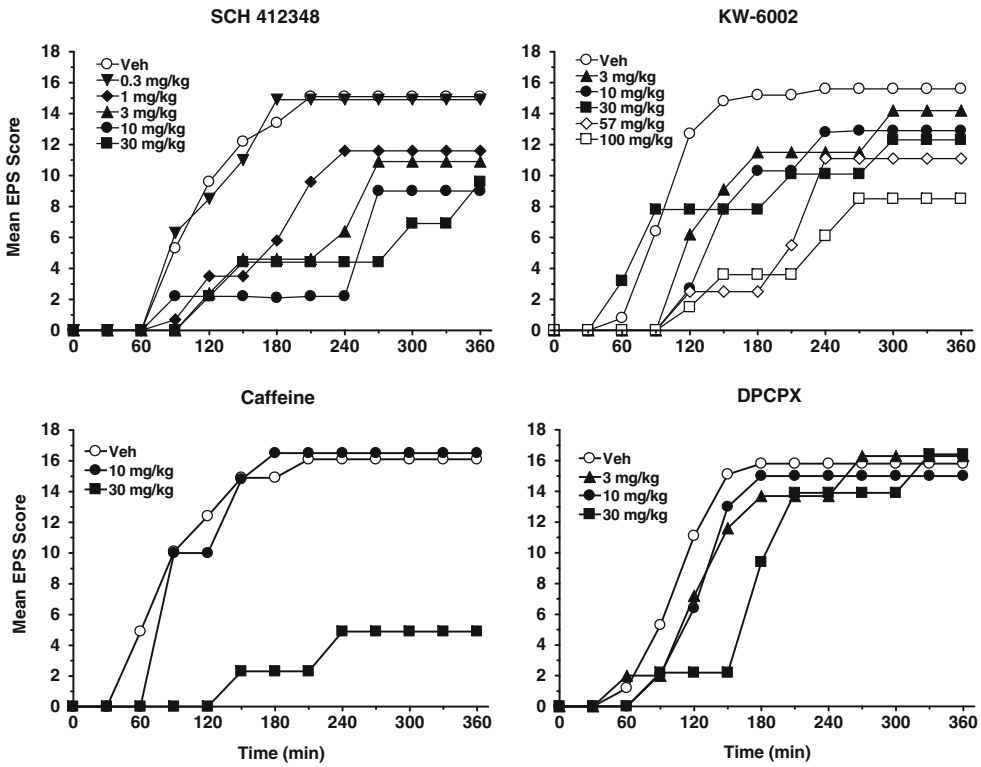

Fig. 1 Time course of haloperidol-induced EPS in sensitized *C. apella* primates following pretreatment with the adenosine A_{2A} receptor antagonists, SCH 412348 (**a**) and KW-6002 (**b**); the A_1/A_{2A} antagonist, caffeine (**c**); and the A_1 antagonist, DPCPX (**d**). Data represent the mean EPS score in six to seven monkeys

coadministration. At this time point, SCH 412348 reduced the mean EPS score although this effect just failed to reach the significance criteria (Fr=10.4, p=0.07). The effect of SCH 412348 was apparent when medians and IQR for doses of 1–30 mg/kg were compared to the vehicle-treated group (Veh) [median (IQR): Veh=15.3 (13.9–16), 0.3 mg/kg=14.5 (13.3–16.25), 1 mg/kg=13.3 (0–16), 3 mg/kg=0 (0–15.5), 10 mg/kg=0 (0–0), 30 mg/kg=0 (0–15.25)]. KW-6002 produced a similar reduction in the EPS score, but again this effect was not significant. However, the effect of KW-6002 was apparent when medians and IQR for doses of 57 and 100 mg/kg were compared to Veh [median (IQR): Veh=15.3 (13.5–16.8), 3 mg/kg=16.1 (0–18.5), 10 mg/kg=15 (0–15.9), 30 mg/kg=13 (0–17.3), 57 mg/kg=0 (0–16.5), 100 mg/kg=0 (0–10.9)]. Caffeine significantly reduced the EPS score (Fr=7.7, p<0.05) at a dose of 30 mg/kg, equivalent to approximately 24 cups of coffee [median (IQR): Veh=16.5 (14.5–17.6), 10 mg/kg=17.3 (15–18), 30 mg/kg=0 (0–0)]. DPCPX had no effect on the EPS score [median (IQR): Veh=15.8 (14.9–17.2), 3 mg/kg=14.5 (14–17.5), 10 mg/kg=14.8 (14–16), 30 mg/kg=15.3 (14.5–17.8)].

An additional observation from these studies was that the onset of EPS was "all-or-nothing" in nature (i.e., animals display either a full EPS episode or no effects) with some adenosine antagonist-treated monkeys showing a delayed profile whereby the onset of EPS occurred 1–2 h later than vehicle-treated controls, while other monkeys remained free of EPS throughout the 6-h observation period. Specifically, as illustrated in Fig. 2, administration of SCH 412348 at doses of 1–30 mg/kg completely blocked the EPS for the entire 6-h test in up to three out of the seven monkeys. Likewise, KW-6002 and caffeine completely blocked the EPS for 6 h in two out of six and five out of seven monkeys, respectively. All seven monkeys treated with DPCPX exhibited full EPS by 210 min after haloperidol administration. We believe that the "all-or-nothing" nature of the response is simply due to the severity of the syndrome once animals are sensitized and that it is difficult to have a partial syndrome.

The apparent delay in EPS onset was analyzed by averaging the onset time for each animal following vehicle or drug treatment. SCH 412348 increased EPS onset time (Fr=24.4, p<0.001) with significant effects at 10 and 30 mg/kg (Fig. 3a). Similarly, KW-6002 increased onset time at all doses compared to Veh (Fig. 3b) with significant increases in onset time at 57 and 100 mg/kg (Fr=13.5, p<0.05). Caffeine also increased the time of EPS onset (Fr=10.6, p<0.01) with a significant effect at the 30-mg/kg dose (Fig. 3c). Interestingly, DPCPX also produced a significant effect on onset time (Fr=14.1, p<0.01) at a dose of 30 mg/kg (Fig. 3d), although the magnitude of the effect was smaller than the other compounds.

Squirrel monkey catalepsy studies

SCH 412348 (10 mg/kg) and caffeine (10 mg/kg; equivalent to approximately eight cups of coffee) significantly

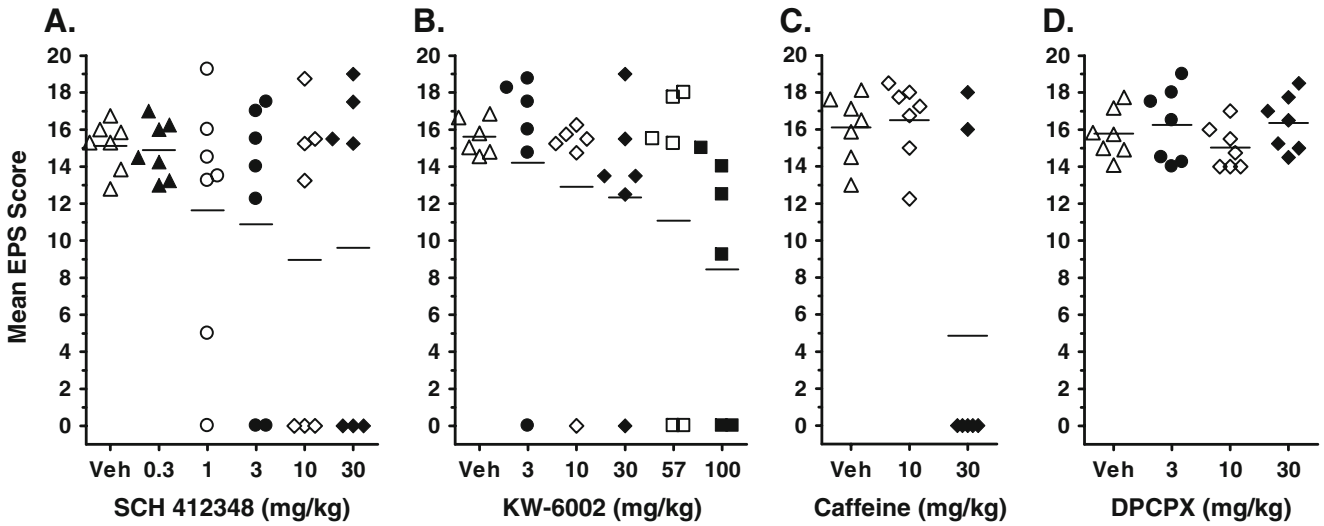

Fig. 2 EPS score for individual *C. apella* monkeys (data points) and the mean EPS score (*horizontal line*) 6 h after treatment with the adenosine A_{2A} receptor antagonists, SCH 412348 (**a**) and KW-6002 (**b**); the A_1/A_{2A} antagonist, caffeine (**c**); and the A_1 antagonist, DPCPX (**d**)

reduced the time spent cataleptic [SCH 412348: $F(3,6)=7.2$, $p<0.05$; caffeine: $F(3,6)=5.3$, $p<0.05$] (Fig. 4). DPCPX had no effect on the cataleptic behavior.

Discussion

Pharmacological blockade of DA D_2 receptors produces therapeutic effects in schizophrenic patients and is a central mechanism underlying the beneficial effects of established antipsychotic drugs (Seeman and Lee 1975). The antipsychotic effects resulting from blocking D_2 receptors are believed to be largely due to reduced activation of the mesolimbic A10 DA pathway connecting the ventral tegmental area (VTA) to the nucleus accumbens within the striatum (Arnt and Skarsfeldt 1998). However, this pharmacological approach of nonselectively blocking dopaminergic receptors can result in prominent side effects characterized as EPS, which is mediated through blockade of other dopaminergic pathways, in particular the nigrostriatal A9 pathway (Crocker and Hemsley 2001).

The *C. apella* monkey model of haloperidol-induced EPS is widely regarded to be a close representative of the syndrome produced by chronic administration of certain

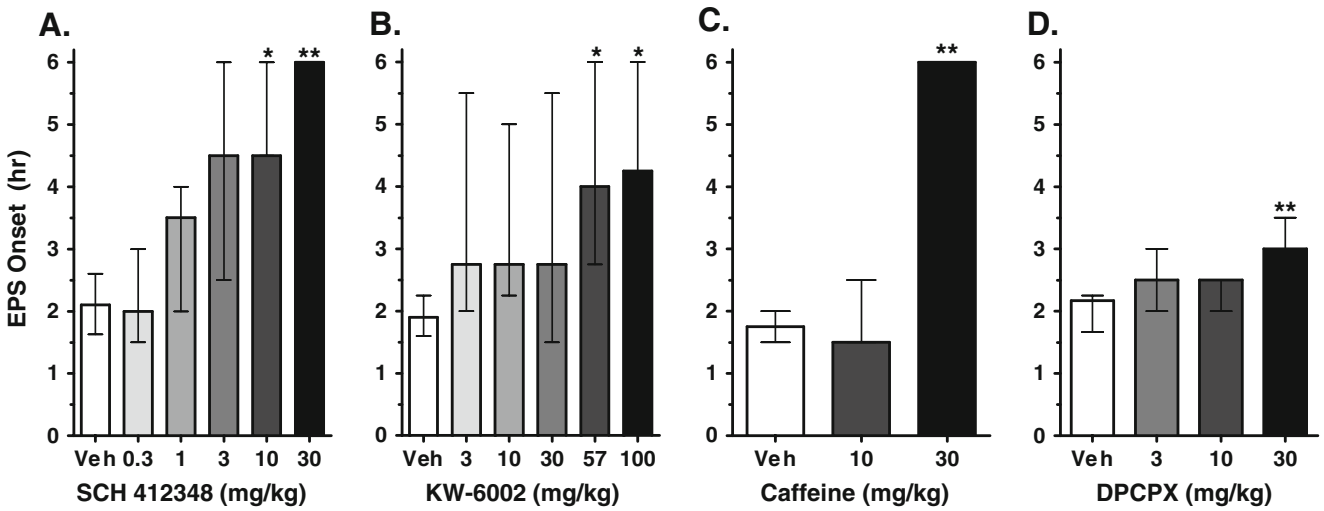

Fig. 3 Time of EPS onset for *C. apella* monkeys treated with the adenosine A_{2A} receptor antagonists, SCH 412348 (**a**) and KW-6002 (**b**); the A_1/A_{2A} antagonist, caffeine (**c**); and the A_1 antagonist, DPCPX (**d**). Data represent the median and IQR for six to seven monkeys. $^{**}p<0.01$; $^{*}p<0.05$ vs. vehicle controls (*Veh*)

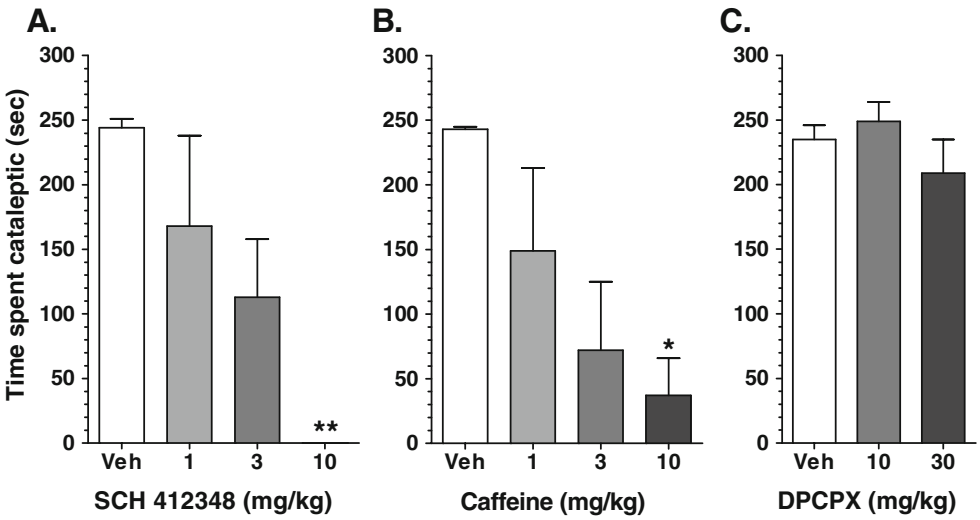

Fig. 4 Effects of the adenosine A_{2A} antagonist, SCH 412348 (**a**); the A_1/A_{2A} antagonist, caffeine (**b**); and the A_1 antagonist, DPCPX (**c**) on haloperidol-induced catalepsy in three squirrel monkeys. $^{**}p<0.01$, $^{*}p<0.05$ vs. vehicle controls (*Veh*)

antipsychotic drugs in humans (Gunne and Barany 1976; Weiss et al. 1977). Although the syndrome varies in nature and severity between monkeys, on the whole, the model encompasses many of the behavioral effects seen in humans following antipsychotic treatment. Consequently, this model has been used to determine the side effect profile of a number of potentially novel antipsychotic drugs, such as dopamine D_1 receptor antagonists, muscarinic antagonists, and cannabinoid CB1 antagonists (Coffin et al. 1989; Casey 1995; Andersen et al. 2003; Madsen et al. 2006). Given the evidence that adenosine antagonists can inversely modulate dopaminergic pathways via the 'indirect' pathway, one goal of these studies was to determine if the administration of adenosine antagonists could attenuate haloperidol-induced EPS. Furthermore, using receptor subtype-specific antagonists, these studies also sought to determine whether the A_1 or A_{2A} receptor subtype is responsible for mediating these effects. To that end, we found that SCH 412348, KW-6002, and caffeine could delay or prevent the onset of EPS as demonstrated by a reduction in the mean EPS score across the colony of monkeys. Given that SCH 412348 and KW-6002 are highly selective for the A_{2A} receptor (Shimada et al. 1997; Neustadt et al. 2007), it seems highly likely that the ability of these compounds to attenuate haloperidol-induced EPS is mediated via the A_{2A} receptor subtype. Since the selective A_1 receptor antagonist, DCPCX, was relatively ineffective in blocking haloperidol-induced EPS, it is also likely that caffeine's efficacy in this model is due to its ability to antagonize the A_{2A} receptor.

SCH 412348 was significantly more potent than KW-6002 in delaying the onset of haloperidol-induced EPS (MED=10 vs. 57 mg/kg). Given that the in vitro potencies of SCH 412348 and KW-6002 at the A_{2A} receptor are similar (0.6 and 2.2 nM, respectively), the shift in potency likely reflects differences in their pharmacokinetic profiles following oral administration. Indeed, our finding that high doses of KW-6002 are required to attenuate EPS is similar to data from Grondin et al. (1999) who demonstrated that KW-6002 attenuated PD-like behaviors in the MPTP-treated cynomolgus monkey at doses of 60–90 mg/kg.

Although DPCPX produced a slight delay in EPS onset at the highest tested dose, the effect was short-lived and all monkeys exhibited full EPS during the 6-h test, unlike the effects of SCH 412348, KW-6002, and caffeine, which were sustained throughout the test in a portion of the colony. The minimal effect of DPCPX may be mediated via the A_{2A} receptor given the modest affinity of DPCPX for the A_{2A} receptor (A_1 Ki=1.2 nM; A_{2A} Ki=163 nM, data not shown).

While there is an abundance of evidence in the rodent supporting the utility of adenosine A_{2A} receptor antagonists to restore the dopaminergic imbalance in movement disorders such as PD, there is limited work in the primate. A second goal of these studies was to expand the primate work by replicating the anticataleptic effects of A_{2A} antagonists in the monkey. For this work, a catalepsy assay was established in the squirrel monkey using a low dose of 0.03 mg/kg haloperidol. This dose of haloperidol resulted in the three monkeys spending 70–80% of the test period cataleptic, yet the cataleptic state was amenable to drug intervention. Studies aimed at blocking the catalepsy demonstrated that both SCH 412348 and caffeine (KW-6002 was not tested due to a lack of compound) attenuated the cataleptic behavior, while the A_1 antagonist, DPCPX, had no effect in this assay. Therefore, these findings suggest that A_{2A} receptor blockade can attenuate haloperidol-induced cataleptic motor impairment in a squirrel monkey, findings consistent with rodent studies (Kanda et al. 1994; Malec 1997; Mandhane et al. 1997; Shiozaki et al. 1999).

Together, the findings from these two studies further support the ability of selective adenosine A_{2A} receptor antagonists to modulate the dopaminergic pathways impli-

cated in disorders such as PD- and antipsychotic-induced EPS. Additionally, these studies provide in vivo data in the primate to support the large body of research in the rodent. Based on the present primate studies, A_{2A} receptor antagonists clearly have potential to treat numerous movement disorders in humans.

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References

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271

Andersen MB, Fink-Jensen A, Peacock L, Gerlach J, Bymaster F, Lundbaek JA, Werge T (2003) The muscarinic M1/M4 receptor agonist xanomeline exhibits antipsychotic-like activity in *Cebus apella* monkeys. Neuropsychopharmacology 28:1168–1175

Arnt J, Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 18:63–101

Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M, Morris MJ, Mouradian MM, Chase TN (2003) Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. Neurology 61:293–296

Cantor CR, Stern MB (2002) Dopamine agonists and sleep in Parkinson's disease. Neurology 58(4 Suppl 1):S71–S78

Casey DE (1995) The effects of D1 (NNC 22-0215) and D2 (haloperidol) antagonists in a chronic double-blind placebo controlled trial in *Cebus* monkeys. Psychopharmacology 121:289–293

Chase TN, Bibbiani F, Bara-Jimenez W, Dimitrova T, Oh-Lee JD (2003) Translating A2A antagonist KW6002 from animal models to parkinsonian patients. Neurology 61(Suppl 6):S107–S111

Coffin VL, Latranyi MB, Chipkin RE (1989) Acute extrapyramidal syndrome in *Cebus* monkeys: development mediated by dopamine D2 but not D1 receptors. J Pharmacol Exp Ther 249:769–774

Coffin VL, McHuch D, Chipkin RE, Barnett A (1992) SCH 39166, a potential antipsychotic drug, does not evoke movement disorders in *Cebus* monkeys. Neurochem Int 20(Suppl):141–145

Correa M, Wisniecki A, Betz A, Dobson DR, O'Neill MF, O'Neill MJ, Salamone JD (2004) The adenosine A2A antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to parkinsonism. Behav Brain Res 148:47–54

Crocker AD, Hemsley KM (2001) An animal model of extrapyramidal side effects induced by antipsychotic drugs: relationship with D2 dopamine receptor occupancy. Prog Neuropsychopharmacol Biol Psychiatry 25:573–590

Driver-Dunckley E, Samanta J, Stacy M (2003) Pathological gambling associated with dopamine agonist therapy in Parkinson's disease. Neurology 61:422–423

Ferre S, Fuxe K, von Euler G, Johansson B, Fredholm BB (1992) Adenosine-dopamine interactions in the brain. Neuroscience 51:501–512

Ferre S, O'Connor WT, Fuxe K, Ungerstedt U (1993) The striopallidal neuron: a main locus for adenosine-dopamine interactions in the brain. J Neurosci 13:5402–5406

Ferre S, Fredholm BB, Morelli M, Popoli P, Fuxe K (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 20:482–487

Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 53:527–552

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 15:133–139

Grondin R, Bedard PJ, Hadj Tahar A, Gregoire L, Mori A, Kase H (1999) Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology 52:1673–1677

Gunne LM, Barany S (1976) Haloperidol-induced tardive dyskinesia in monkeys. Psychopharmacology 50:237–240

Hauber W, Neuscheler P, Nagel J, Muller CE (2001) Catalepsy induced by a blockade of dopamine D1 or D2 receptors was reversed by a concomitant blockade of adenosine A(2A) receptors in the caudate-putamen of rats. Eur J Neurosci 14:1287–1293

Hauser RA, Hubble JP, Truong DD, Istradefylline US-001 Study Group (2003) Randomized trial of the adenosine A(2A) receptor antagonist istradefylline in advanced PD. Neurology 61:297–303

Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 431:331–346

Jacobson KA, Gao ZG (2006) Adenosine receptors as therapeutic targets. Nat Rev Drug Discov 5:247–264

Jenner P (2005) Istradefylline, a novel adenosine A2A receptor antagonist, for the treatment of Parkinson's disease. Expert Opin Investig Drugs 14:729–738

Johansson B, Georgiev V, Fredholm BB (1997) Distribution and postnatal ontogeny of adenosine A2A receptors in rat brain: comparison with dopamine receptors. Neuroscience 80:1187–1207

Kafka SH, Corbett R (1996) Selective adenosine A2A receptor/dopamine D2 receptor interactions in animal models of schizophrenia. Eur J Pharmacol 295:147–154

Kanda T, Shiozaki S, Shimada J, Suzuki F, Nakamura J (1994) KF17837: a novel selective adenosine A2A receptor antagonist with anticataleptic activity. Eur J Pharmacol 256:263–268

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, Kuwana Y, Jenner P (1998) Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 43:507–513

Kurokawa M, Koga K, Kase H, Nakamura J, Kuwana Y (1996) Adenosine A2a receptor-mediated modulation of striatal acetylcholine release in vivo. J Neurochem 66:1882–1888

Latini S, Pazzagli M, Pepeu G, Pedata F (1996) A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. Gen Pharmacol 27:925–933

Lohse MJ, Klotz KN, Lindenborn-Fotinos J, Reddington M, Schwabe U, Olsson RA (1987) 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)—a selective high affinity antagonist radioligand for A1 adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol 336:204–210

Madsen MV, Peacock L, Werge T, Andersen MB (2006) Effects of the cannabinoid CB1 receptor agonist CP55,940 and antagonist SR141716A on d-amphetamine-induced behaviours in *Cebus* monkeys. J Psychopharmacol 20:622–628

Malec D (1997) Haloperidol-induced catalepsy is influenced by adenosine receptor antagonists. Pol J Pharmacol 49:323–327

Mandhane SN, Chopde CT, Ghosh AK (1997) Adenosine A2 receptors modulate haloperidol-induced catalepsy in rats. Eur J Pharmacol 328:135–141

Marsden CD (1994) Problems with long-term levodopa therapy for Parkinson's disease. Clin Neuropharmacol 17(Suppl 2):S32–S44

Mayfield RD, Larson G, Orona RA, Zahniser NR (1996) Opposing actions of adenosine A2a and dopamine D2 receptor activation on GABA release in the basal ganglia: evidence for an A2a/D2 receptor interaction in globus pallidus. Synapse 22:132–138

Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA (2007) Role of adenosine A2A receptors in parkinsonian motor impairment and l-DOPA-induced motor complications. Prog Neurobiol 83:293–309

Mori A, Shindou T (2003) Modulation of GABAergic transmission in the striatopallidal system by adenosine A2A receptors: a potential mechanism for the antiparkinsonian effects of A2A antagonists. Neurology 61(Suppl 6):S44–S48

Neustadt BR, Hao J, Lindo N, Greenlee WJ, Stamford AW, Tulshian D, Ongini E, Hunter J, Monopoli A, Bertorelli R, Foster C, Arik L, Lachowicz J, Ng K, Feng KI (2007) Potent, selective, and orally active adenosine A(2A) receptor antagonists: arylpiperazine derivatives of pyrazolo[4,3-e]-1,2,4-triazolo [1,5-c]pyrimidines. Bioorg Med Chem Lett 17:1376–1380

Obeso JA, Grandas F, Vaamonde J, Luquin MR, Artieda J, Lera G, Rodriguez ME, Martinez-Lage JM (1989) Motor complications associated with chronic levodopa therapy in Parkinson's disease. Neurology 39(Suppl 2):11–19

Parent A, Cicchetti F (1998) The current model of basal ganglia organization under scrutiny. Mov Disord 13:199–202

Pinna A, Fenu S, Morelli M (2001) Motor stimulant effects of the adenosine A(2A) receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse 39:233–238

Pinna A, Pontis S, Borsini F, Morelli M (2007) Adenosine A2A receptor antagonists improve deficits in initiation of movement and sensory motor integration in the unilateral 6-hydroxydopamine rat model of Parkinson's disease. Synapse 61:606–614

Pollack AE, Harrison MB, Wooten GF, Fink JS (1993) Differential localization of A2a adenosine receptor mRNA with D1 and D2 dopamine receptor mRNA in striatal output pathways following a selective lesion of striatonigral neurons. Brain Res 631:161–166

Popoli P, Frank C, Tebano MT, Potenza RL, Pintor A, Domenici MR, Nazzicone V, Pezzola A, Reggio R (2003) Modulation of glutamate release and excitotoxicity by adenosine A2A receptors. Neurology 61(Suppl 6):S69–S71

Richardson PJ, Kase H, Jenner PG (1997) Adenosine A2A receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci 18:338–344

Rosenzweig-Lipson S, Bergman J (1994) Catalepsy-associated behavior induced by dopamine D1 receptor antagonists and partial dopamine D1 receptor agonists in squirrel monkeys. Eur J Pharmacol 260:237–241

Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J (1998) Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. J Comp Neurol 401:163–186

Schiffmann SN, Vanderhaeghen JJ (1993) Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J Neurosci 13:1080–1087

Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277–292

Seeman P, Lee T (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 188:1217–1219

Shimada J, Koike N, Shiozaki S, Yanagawa K, Kanda T, Kobayashi H, Ichimura M, Nakamura J, Kase H, Suzuki F (1997) Adenosine A2A antagonists with potent anti-cataleptic activity. Bioorg Med Chem Lett 7:2349–2352

Shiozaki S, Ichikawa S, Nakamura J, Kitamura S, Yamada K, Kuwana Y (1999) Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology 147:90–95

Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999) Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 59:355–396

Weiss B, Santelli S, Lusink G (1977) Movement disorders induced in monkeys by chronic haloperidol treatment. Psychopharmacology 53:289–293