Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 83 (2006) 114–121
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of the A_{2A} adenosine receptor antagonist KW6002 in the nucleus accumbens in vitro and in vivo

L.K. Harper [a,1], S.R. Beckett [a], C.A. Marsden [a], A.C. McCreary [b], S.P.H. Alexander [a,*]

[a] School of Biomedical Sciences and Institute of Neuroscience, University of Nottingham Medical School, Nottingham, UK
[b] Solvay Pharmaceuticals Research Laboratories, C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands

Received 30 August 2005; received in revised form 25 November 2005; accepted 20 December 2005
Available online 31 January 2006

Abstract

In this study, we have used the selective A_{2A} adenosine receptor antagonist KW6002 to investigate the function of A_{2A} receptors in the Lister hooded rat nucleus accumbens in vitro and in vivo. Radioligand binding studies confirmed a greater than 50-fold selectivity of KW6002 for A_{2A} receptors compared to A_1 receptors. Release of [^{3}H]-dopamine from nucleus accumbens slices in vitro was almost doubled in the presence of 300 nM KW6002, while GABA release was inhibited by approximately one third. In vivo, intraperitoneal administration of KW6002 (4 mg kg^{-1}) increased dopamine overflow almost 4-fold in the nucleus accumbens. In behavioural testing, KW6002 elicited place preference and increased locomotor activity at 1, 2 and 4 mg kg^{-1}. Taken together, these results suggest a role for tonic activation of A_{2A} adenosine receptors in reward-related phenomena.

Keywords: A_{2A} adenosine receptors; Nucleus accumbens; Dopamine release; Conditioned place preference; Reward

1. Introduction

A_{2A} adenosine receptor modulation in basal ganglia circuitry has been reported to influence motor functions in behavioural studies. Agonists at the receptor depress motor activity in rodents and primates (Ferre et al., 1991; Morelli et al., 1994; Kanda et al., 1998), while A_{2A} receptor blockade produces motor stimulant effects. A_{2A} receptor antagonism potentiates dopaminergic drug-induced rotation in 6-hydroxydopamine (6-OHDA)-treated rats (Pollack and Fink, 1996; Fenu et al., 1997) and reversal of catalepsy induced by reserpine or haloperidol (Kanda et al., 1994) as well as motor impairment in D_2 receptor knockout mice (Aoyama et al., 2000; Chen et al., 2001).

The first orally active A_{2A} adenosine receptor antagonist, KW6002, was developed in the late 1990s (Shimada et al., 1997) and displays high affinity for rat caudate-putamen A_{2A} receptors (K_i 2.2 nM) with a 68-fold lower affinity for rat forebrain A_1 receptors (Shimada et al., 1997). Following oral administration, KW6002 was found to be distributed selectively to the caudate-putamen and nucleus accumbens (NAc) at doses of 0.1 and 0.3 mg kg^{-1}, with density values in these areas being around 2.5 times higher than in frontal cortex. This ratio, however, decreased to around 1.6 at a dose of 3 mg kg^{-1} and the compound was distributed more widely in the brain (Aoyama et al., 2002). KW6002 has been reported to be active in experimental models of Parkinson's disease and locomotor dysfunction. It improves motor function in drug-induced catalepsy models in mice, 6-OHDA-treated rats and MPTP-treated monkeys (Shiozaki et al., 1999; Kanda et al., 2000; Koga et al., 2000), indicating that the compound may be a useful non-dopaminergic drug for the treatment of Parkinson's disease (Richardson et al., 1997; Kuwana et al., 1999). Indeed, in pre-clinical and clinical assessments of Parkinson's disease, KW6002 has shown efficacy in symptom alleviation (Bara-Jimenez et al., 2003; Hauser et al., 2003; Lundblad et al., 2003; Chen et al., 2003; Kase et al., 2003; Pierri et al., 2005). As an inevitable consequence of systemic administration, KW6002 (as with other potential treatments for Parkinson's disease) will distribute to both caudate-putamen and NAc, at which distinct behavioural outcomes would be expected.

* Corresponding author. Tel.: +44 115 9709 474; fax: +44 115 9709 259.
E-mail address: steve.alexander@nottingham.ac.uk (S.P.H. Alexander).
[1] Present address: Department of Pharmacology and Experimental Therapeutics, Loyola University Chicago, School of Medicine, Maywood, IL 60153-5515, USA.

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.12.014

In a microdialysis study using 6-OHDA-lesioned rats, KW6002 decreased GABA levels in the globus pallidus immediately following treatment (Ochi et al., 2000). The effects of this antagonist on extracellular dopamine, however, have not been measured in vivo. An increase in NAc extracellular dopamine is believed to be one of the main mechanisms in the rewarding and motor stimulating effects of psychostimulants. Solinas et al. (2002) reported caffeine (which is a poorly-selective adenosine receptor antagonist in vitro) induced dopamine and glutamate release in the NAc (Solinas et al., 2002), while Acquas et al. (2002) reported no effect of either caffeine or the A_{2A} selective antagonist SCH58261 on dopamine release in this region. SCH58261 has also been shown to increase extracellular glutamate levels in the dopamine-denervated rat (Corsi et al., 2003), but an earlier study demonstrated an increase in glutamate release using the A_{2A} agonist CGS21680 (Popoli et al., 1995).

Given the potential for KW6002 and other A_{2A} receptor antagonists as a therapeutic approach in the treatment of disorders such as Parkinson's disease (primarily through actions in the caudate-putamen), an investigation of the effects of this A_{2A}-selective antagonist on dopamine release in vitro and in vivo in the NAc, and its effects in a model of reward-related behaviour is important, as effects in this brain region may help explain both potential clinical benefit and side effects. Some of these results have been presented in preliminary form elsewhere (Harper et al., 2003).

2. Materials and methods

2.1. Animals

Lister hooded rats (male, 280–330 g) were bred at the BMSU, University of Nottingham. Animals were group-housed (3–4 per cage) with food and water available ad libitum. Lighting was provided from 0700 to 1900 h, with all procedures carried out between 0800 and 1700 h, according to UK Home Office Regulations (Project License PP 40/1955).

2.2. Drugs and materials

KW6002 was synthesised in house at Solvay Pharmaceuticals and was initially dissolved to a concentration of 0.01 M in DMSO and stored at −20 °C, protected from light, for up to one week. [^{3}H]-ZM241385 (629 GBq mmol^{-1}) was a kind gift from Tocris Cookson, Avonmouth, UK, while [^{3}H]-DPCPX (4040 GBq mmol^{-1}) was obtained from GE NEN DuPont, Herts, UK. [^{3}H]-Dopamine and [^{3}H]-GABA were obtained from Amersham Pharmacia Biotech, Herts, UK. Unless specified otherwise, all other chemicals were obtained from Sigma Chemical Company, Dorset, UK.

2.3. In vitro measurement of receptor binding and transmitter release

Brain regions were dissected rapidly on ice after decapitation, with particulate preparations produced as previously described (Alexander and Millns, 2001). [^{3}H]-ZM241385 and [^{3}H]-DPCPX binding at 0.3–0.5 nM were assessed in the presence of 1 U/ml adenosine deaminase in a final assay volume of 0.25 ml (Alexander and Millns, 2001).

[3H]-Dopamine and [3H]-GABA release from NAc slices (350×350 μm) was conducted as previously described (Cadogan et al., 1997). Slices were incubated with [^{3}H]-dopamine (370 Bq ml^{-1}, in the presence of 10 μM pargyline) or [^{3}H]-GABA (370 Bq ml^{-1}, in the presence of 50 μM amino oxyacetic acid), at 37 °C for 15 min before being washed and transferred to a Brandel Suprafusion Unit. The slices were then superfused with fresh Krebs for 45 min prior to sample collection. 3 min samples were collected (at a rate of 0.5 ml min^{-1}) in an automatic collection tray beginning at t=0. Transmitter release was electrically stimulated at t=15 min (S1) and t=42 min (S2) for 3 min with 5 ms biphasic pulses, 100 mA in amplitude at a frequency of 0.5 Hz. Test drugs were added to the suprafusion fluid at t=24 min and removed at t=45 min. In experiments examining [^{3}H]-GABA release, 10 μM NO711 (1-(2-[{(diphenylmethylene)imino}oxy]ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride) and 100 μM β-alanine were used throughout the release phase to prevent re-uptake of GABA. The amount of radioactivity present in the fractions collected was then determined by liquid scintillation spectrometry. Data are expressed as ratios of [^{3}H]-DA and [^{3}H]-GABA released during the second and first periods of stimulation (S2/S1) or that released in the absence of stimulation (C2/C1) from at least 3 separate experiments. The three values prior to first and second stimulations were used to calculate C1 and C2. S1 and S2 values were taken at the peaks of stimulus-evoked release.

2.4. In vivo measurement of nucleus accumbens extracellular dopamine

In vivo microdialysis to determine extracellular dopamine levels in Lister hooded rats was conducted as previously described (Fulford and Marsden, 1998). Concentric microdialysis probes (300 μm o.d., 220 μm i.d.) were constructed from semi-permeable renal dialysis tubing (polyacrylonitrile/sodium methanylsulphonate copolymer, Hospal UK) with a molecular weight cut off point of 20 kDa. Recovery through the membrane was measured in vitro using a 10 nM dopamine standard solution. Perchloric acid (PCA) (0.4%) was included in the standard solution as an antioxidant. Probes were perfused at 1.2 μl min^{-1} at a temperature of 22–23 °C. Mean recovery was 23±1%.

Animals were anaesthetised with an isoflurane/N_2O mixture (N_2O 1 l min^{-1}:O_2 0.5 l min^{-1}; 4% isoflurane for induction; 1.5–2% for maintenance) and placed in a stereotaxic head frame (Kopf Instruments, USA) using blunt ear bars. The incisor bar was positioned at −3.3 mm below intraaural zero such that the skull was horizontal between lambda and bregma (Paxinos and Watson, 1997). The skull was exposed to allow implantation of a microdialysis probe connected to a liquid swivel/counter-balanced arm system (Medical Engineering Unit, University of Nottingham) and perfused with artificial CSF (mM: NaCl

140.0, KCl 3.0, $CaCl_2$ 2.5, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.27, glucose 7.2, pH 7.4). The dialysis probe was inserted into the nucleus accumbens (coordinates: AP −1.2, ML ±1.6, DV −8.0 relative to bregma, (Paxinos and Watson, 1997). The probe was anchored using two skull screws and fixed with dental cement. The incision was sutured and treated (Nobocutaine), allowing animals to recover for 44 h prior to sampling.

Dialysis samples were collected at 20 min intervals into 0.2 ml tubes containing 5 μl perchloric acid and immediately snap frozen and stored at −70 °C until assay. A total of 16 samples were collected for each animal. On completion of the experiment, animals were killed by anaesthetic overdose (Euthatal 10 mg kg^{-1}) and the probes perfused with pontamine sky blue solution. Brains were then removed and fixed in 30% formaldehyde, prior to histological verification of location.

Dopamine in dialysates was quantified using reverse-phase ion-pair HPLC with electrochemical detection. Fractions were separated using a 100×2.0 mm reverse-phase column with a 3 μm C18 Nucleosil packing (Phenomenex, UK) and mobile phase (1 mM EDTA, 1.2 mM 1-octane-sulfonic acid, 0.1 M $NaH_2PO_4 \cdot 2H_2O$, with 10% methanol, at pH 4.0–4.1) delivered at a flow rate of 0.2 ml min^{-1}. Peaks for dopamine were identified by comparison with retention times of dopamine standard solutions. The relationship between dopamine concentration and detector response was linear.

Following collection of 6 basal samples, one group of rats (n=6) received 4 mg kg^{-1} KW6002 (dissolved 1:1:18 in DMSO:Cremophor EL:saline) by i.p. injection. A control group (n=6) received an i.p. injection of vehicle at the same time point. In controls, immediately following collection of sample no. 10, the probes were switched to a perfusion fluid containing 1 μM tetrodotoxin in aCSF.

2.5. Conditioned place preference

Conditioned place preference was determined using a previously described methodology (Cheer et al., 2000). Briefly, the apparatus consisted of two perspex compartments (30×30×40 cm each), separated by a removable partition. Each compartment had different visual cues in the form of thick (2.5 cm) or thin (1 cm) vertical black and white stripes. The floor was uniformly smooth in both compartments. Prior to starting the experiment, animals were handled once daily for 7 days. During the habituation phase, the partition was removed and the animals were placed in the middle of the apparatus and allowed to explore for 10 min. This was done for each animal over three consecutive days and the amount of time spent in each compartment was averaged for this phase. The animals displayed no significant preference for either compartment (data not shown). Animals were randomly assigned to groups (n=6/group) for a conditioning phase which consisted of three pairings of vehicle or 1, 2 or 4 mg kg^{-1} KW6002 (dissolved 1:1:18 in DMSO:Cremophor EL:saline) by i.p. injection with one compartment, alternated with three pairings of corresponding vehicle with the other compartment. Control groups received vehicle throughout. Rats were placed in their respective compartments with the partition in place for 10 min, 30 min after injection (during which time, animals were placed in a neutral tub).

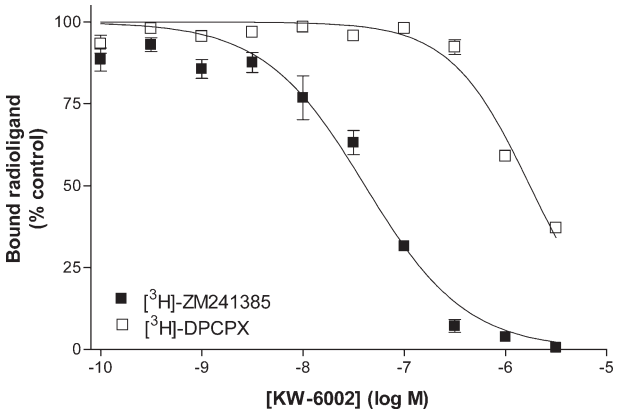

Fig. 1. Competition analysis of [^{3}H]-ZM241385 and [^{3}H]-DPCPX binding to caudate-putamen membranes from male Lister hooded rats. Data are means and SEM of triplicate estimates. Where error bars are not shown, they are smaller than the symbol.

The conditioning phase and test session were separated by 48 h to allow the animals to form the necessary drug-environment associations. During the test phase, the partition was removed and the animals placed in the middle of the two compartments and the time spent in each compartment was measured. A camera mounted on the ceiling perpendicularly to the CPP apparatus recorded all sessions. The time spent in each compartment and distanced travelled during the habituation and test sessions was analysed by a computer-automated tracking system (Ethovision™).

2.6. Data analysis

Data were analysed for statistical significance using the computer program Prism for two-way ANOVA, repeated measures ANOVA and post hoc Dunnett's test and Student's t-test. $P<0.05$ was taken as a threshold of statistical significance.

3. Results

3.1. In vitro receptor binding

KW6002 was found to have a greater than 50-fold selectivity for A_{2A} receptors over A_1 receptors in membranes from Lister hooded male rat brains. pK_i values of 7.63±0.09 (Hill slope −0.91±0.15) and 5.88±0.01 (Hill slope −1.10±0.05) were estimated for A_{2A} and A_1 receptor binding, respectively (Fig. 1). These values are equivalent to K_i values of 24 and 1320 nM, respectively, giving a selectivity ratio at A_{2A} receptors of 56.

3.2. In vitro neurotransmitter release

[^{3}H]-Dopamine release from NAc slices was maintained over the control period (C2/C1 97±1%) and was reduced

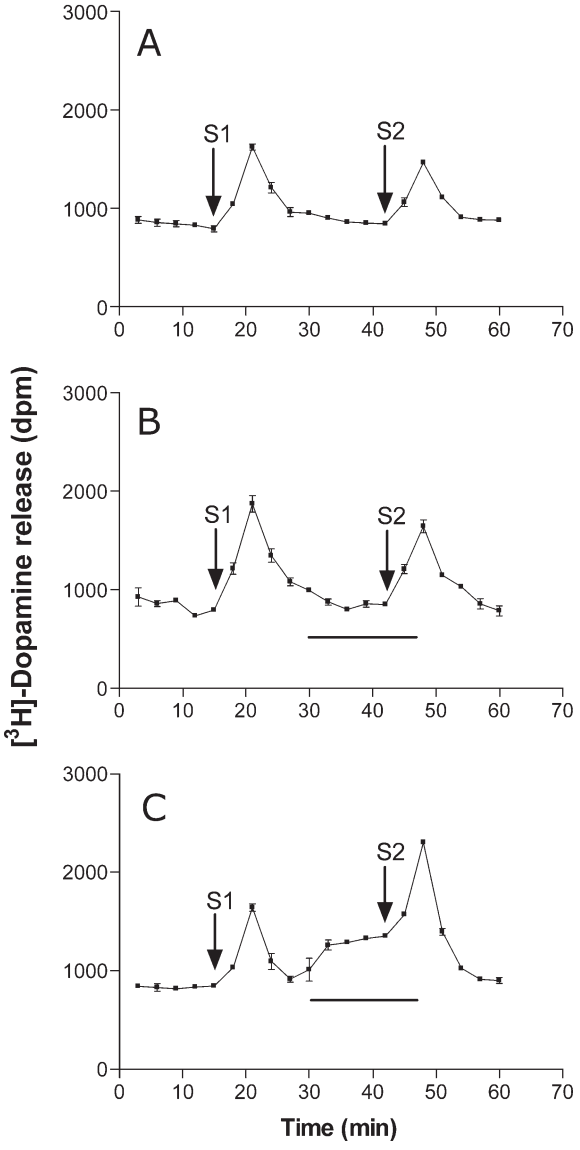

Fig. 2. [^{3}H]-dopamine release from rat NAc slices in A) control conditions, B) presence of 30 nM KW6002 and C) presence of 300 nM KW6002 prior to S2, indicated by the horizontal bars. Data are means±SEM of triplicate estimates from an individual experiment representative of three. Where error bars are not visible, they are smaller than the symbol.

during the second period of stimulation (S2/S1 79±2%). The presence of 30 nM KW6002 in the suprafusion buffer had no significant effect on [^{3}H]-dopamine release in control or stimulation phases. Addition of 300 nM KW6002, however, caused a significant increase in [^{3}H]-dopamine release in both the absence and presence of electrical stimulation (C2/C1 126±4%, $P<0.001$; S2/S1 139±5%, $P<0.001$) (Fig. 2).

[^{3}H]-GABA release from NAc slices was also maintained over the control period (C2/C1 86±3%) and reduced during the second period of stimulation (S2/S1 73±4%). Inclusion of 300 nM KW6002 in the suprafusion fluid caused a significant decrease in [^{3}H]-GABA release in both control and stimulated phases (C2/C1 62±4%, $P<0.01$; S2/S1 55±6%, $P<0.01$; Fig. 3).

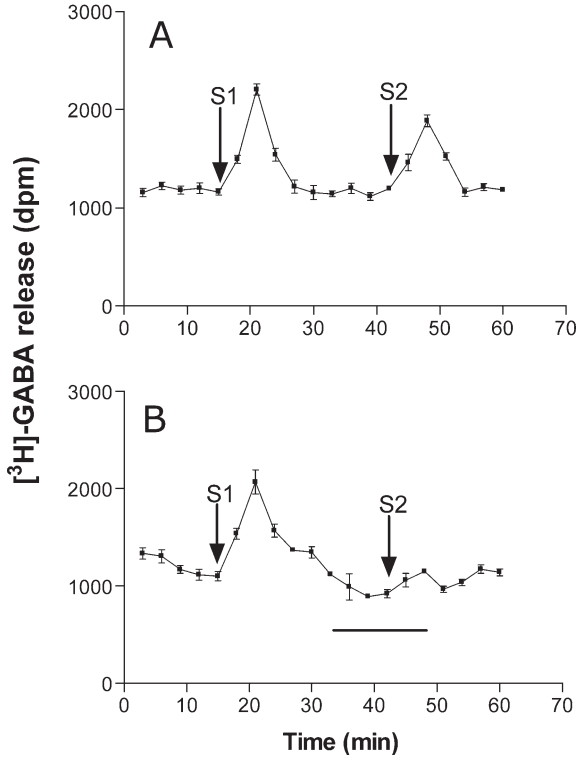

Fig. 3. [^{3}H]-GABA release from rat NAc slices in A) control conditions and B) the presence of 300 nM KW6002 prior to S2, indicated by the horizontal bars. Data are means±SEM of triplicate estimates from an individual experiment representative of three. Where error bars are not visible, they are smaller than the symbol.

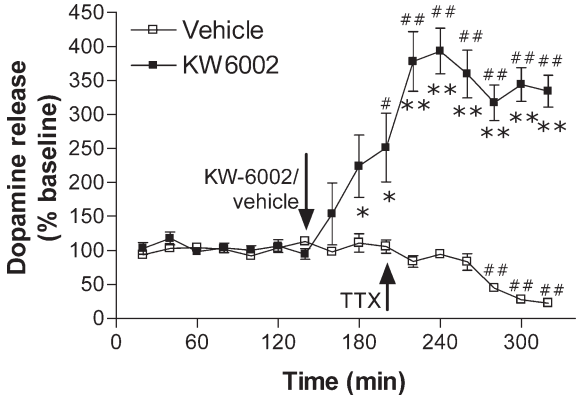

Fig. 4. Effect of 4 mg kg^{-1} KW6002 on accumbens extracellular dopamine level. Basal dopamine level was measured in accumbens perfusate for 120 min, at which time drug-treated animals received KW6002 and control animals received vehicle by i.p. injection. Data are means±SEM for control and drug-treated groups ($n=6$/group), uncorrected for recovery and normalised for graphical purposes to the mean dopamine levels for the control group prior to drug/vehicle addition (20–120 min collection period). Where error bars are not visible, they are smaller than the symbol. Data were analysed for statistical significance using repeated measures ANOVA, comparing dopamine levels at 140–320 min to the mean dopamine level for 20–120 min (control group $F=22.64$, $df=5, 50$; KW6002 group $F=10.45$, $df=5, 50$; $^{\#}P<0.05$, $^{\#\#}P<0.01$). A Students' unpaired t-test also allowed comparison of vehicle group compared to the group receiving KW6002 ($^{*}P<0.05$, $^{**}P<0.001$).

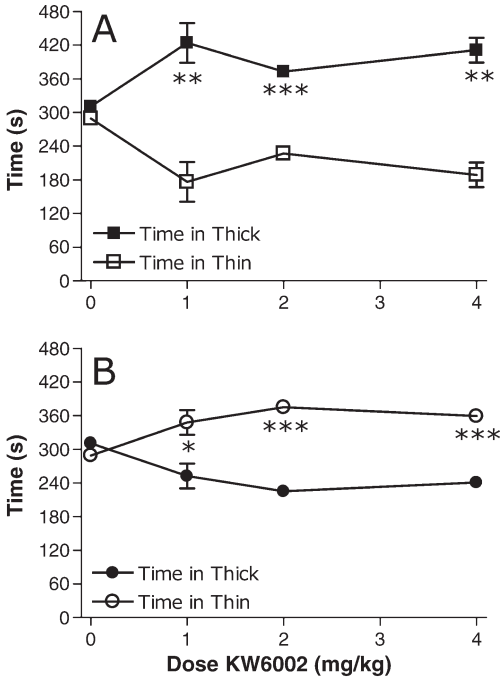

Fig. 5. Effect of KW6002 in the conditioned place preference (CPP) paradigm. Animals were administered KW6002 on three consecutive conditioning days in a CPP chamber with thick- (A) or thin-striped (B) walled compartments. The amount of time spent by animals in each compartment (thick stripes or thin stripes) is shown on the experimental day, 48 h after the last conditioning period. Data were analysed using Student's *t*-test to show differences in time spent in thick or thin-striped compartments (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

3.3. In vivo microdialysis of extracellular dopamine levels

No significant difference in extracellular dopamine between groups was observed prior to drug injection. Following i.p. injection of 4 mg kg^{-1} KW6002, however, extracellular dopamine was significantly increased in animals receiving the drug compared to controls (Fig. 4). A significant decrease in extracellular dopamine levels in control animals was observed following addition of 1 μM tetrodotoxin to the aCSF, compared to control values prior to tetrodotoxin addition (Fig. 4).

3.4. Conditioned place preference

While saline-treated controls displayed no significant preference for either compartment (Fig. 4), KW6002-treated animals spent significantly more time in the drug-paired compartment at all three doses (Fig. 5). During the conditioning phase, it was observed that administration of KW6002 increased locomotor activity significantly compared to controls (Fig. 6).

4. Discussion

In this study, we report that the A_{2A} adenosine receptor regulates extracellular dopamine levels in vitro and in vivo, and has a role in reward related behaviours.

In preliminary studies to confirm the selectivity of KW6002, we observed high affinity for Lister hooded rat caudate-putaman A_{2A} receptors, with a 56-fold higher selectivity for A_{2A} versus A_1 receptors, in accordance with other reports of KW6002 selectivity (Shimada et al., 1997; Harvey et al., 2001). The affinity of KW6002 for A_{2A} receptors in this study is slightly lower than that reported for this antagonist previously (2.2 nM (Shimada et al., 1997); 11 nM (Harvey et al., 2001)). It is interesting to note that binding of the radioligand [^{3}H]-ZM241385 to rat caudate-putamen preparations also appears to yield a wide range of K_d values from 0.21 nM (Lopes et al., 1999) to 0.84 nM (Alexander and Millns, 2001), up to 2.7 nM (Harvey et al., 2001). Whether these differences results from differences in assay conditions or strain of rat remains to be ascertained. Based on the pK_i values obtained in the current investigation, it was calculated that a concentration of 30 nM KW6002 would occupy 55% of A_{2A} receptors and 2% of A_1 receptors, while 300 nM would occupy 92% and 18%, respectively.

An increase in basal and electrically-evoked dopamine release in the presence of 300 nM KW6002 was observed in NAc slices in vitro, indicating the A_{2A} receptor to be a likely target for regulation of accumbens dopamine. We have observed a similar effect of the same concentration of SCH58261 (Harper et al., 2002); a concentration which was also found to be effective as an antagonist in functional assays of A_{2A} receptors, but had limited effects on A_1 receptor and no effect on A_{2B} receptors (Zocchi et al., 1996). Given that KW6002 was able to increase dopamine release at a concentration which would have been likely to block over 90% of A_{2A} receptors, with less than 20% blockade of A_1 receptors, the likely mechanism of action is through A_{2A} receptors. Since the lower concentration of KW6002 (30 nM) failed to have any effect on basal or electrically stimulated dopamine release in the NAc, it is possible that the A_1 receptor may also have a role in regulation of dopamine release in the NAc, since this concentration was calculated to

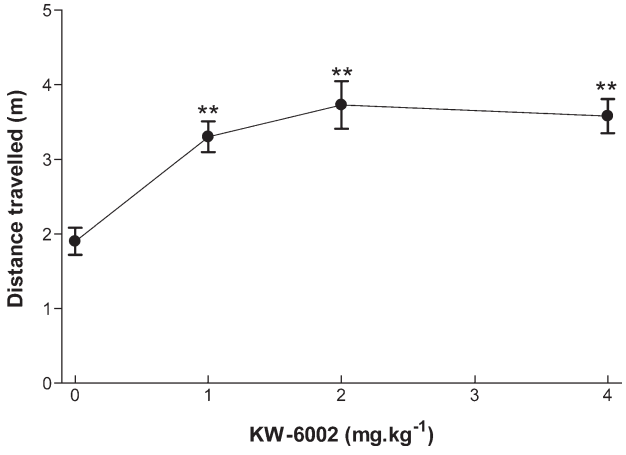

Fig. 6. Effect of KW6002 on locomotor activity. Data are means ± SEM distance travelled in response to KW6002 or vehicle injection for each group ($n=4$/group) in a 10 min period, 30 min following drug administration from the third conditioning period of CPP. Data were analysed using repeated measures ANOVA ($F=12.16$, $df=3, 12$) with Dunnett's post hoc test (**$P<0.01$ versus control).

have negligible A_1 occupancy with 50% A_{2A} occupancy. A role for the A_1 receptor in dopamine release in the NAc has been proposed by Solinas et al. (2002) following their observation that the ability of caffeine to increase extracellular dopamine was reproduced by an A_1 selective antagonist but not by an A_{2A} selective antagonist (Solinas et al., 2002). There is also recent evidence of a facilitatory interaction between A_1 and A_{2A} receptors in the caudate-putamen and NAc (Karcz-Kubicha et al., 2003). It should be borne in mind, however, that the affinities estimated by radioligand binding to cell-free preparations reflect steady state concentrations of the drug, while 300 nM KW6002 appears to evoke an increase in basal [^{3}H]-dopamine release almost immediately upon interaction with NAc tissue slices (Fig. 2C). Although we have not determined the on-rate of KW6002 to occupy A_1 or A_{2A} receptors, our previous studies with [^{3}H]-ZM241385 suggested a rapid equilibration of the radioligand at 37 °C to particulate preparations from the rat caudate-putamen, with a $t_{1/2}$ of 0.27 min (Alexander and Millns, 2001). In slices of pig caudate nucleus incubated at 37 °C in Krebs' solution, we have observed much slower accumulation of 0.4 nM [^{3}H]-ZM241385 ($t_{1/2}$ of 2.3 ± 0.3 min, Alexander, unpublished observations). We assume that this reduced rate of equilibration is partly due to diffusion limitations in intact slice preparations, but it is also possible that endogenous adenosine levels in intact slices (compared to the presence of adenosine deaminase in the binding assay) may contribute. It seems likely, therefore, that the slowed on-rate of antagonist binding in intact tissue slices may well underlie the apparently low potency of KW6002 when compared to receptor occupancy in cell-free preparations from the same tissue.

The observation that KW6002 inhibits GABA release in basal and stimulated conditions supports the suggestion that A_{2A} adenosine receptors in the rat NAc inhibit dopamine release via presynaptic modulation of GABA release. This is in agreement with our previous observations (Harper et al., in preparation), and parallels findings from studies of A_{2A} receptor enhancement of GABA release in rat caudate-putamen and globus pallidus (Mayfield et al., 1993, 1996; Ochi et al., 2000). However, these results contrast with other reports which indicate an A_{2A} receptor inhibition of GABA release in rat caudate-putamen preparations (Kurokawa et al., 1994; Kirk and Richardson, 1994, 1995). Our preliminary findings suggest that an A_{2A} antagonist (SCH58261, data not shown) is able to enhance dopamine release in the caudate-putamen of Lister hooded rats, which suggests that our observations are consistent in the two different brain regions and that the difference in observations is not a consequence of regional variation.

In vivo microdialysis is a useful technique for measuring extracellular levels of neurotransmitters, and allows for assessment of drug effects on transmitter release in the awake, freely moving animal. In this study, an increase in extracellular dopamine levels in the NAc was observed following i.p. injection of the A_{2A} receptor antagonist KW6002. The ability of tetrodotoxin to decrease dopamine overflow substantially indicates that activity, at least in those control animals which received tetrodotoxin, is neuronal. It is likely, although not certain since we did not examine the effects of tetrodotoxin on dopamine levels in KW6002-exposed animals, that the evoked increase in dopamine levels observed with KW6002 is also neuronal.

Caffeine, a non-selective adenosine receptor antagonist, has previously been shown to induce dopamine release in the rat NAc (Solinas et al., 2002), while the A_{2A} selective antagonist SCH58261 did not affect extracellular dopamine release at motor stimulant doses. In another study, neither caffeine nor SCH58261 elicited an increase in NAc dopamine release (Acquas et al., 2002).

The present study demonstrates the effect of A_{2A} adenosine receptor antagonism in a behavioural model of reward. KW6002 was found to produce CPP at all doses tested, in agreement with previous observations for CGS15943 (Harper et al., in preparation). No clear dose-dependency was observed using KW6002, with animals displaying a similar preference for the drug-paired environment at all doses. CGS15943 induced CPP was dose-dependent, however, with animals that received 4 mg kg^{-1} CGS15943 spending more time in the drug-paired environment then animals that received 1 or 2 mg kg^{-1} (Harper et al., in preparation). The discrepancy may be attributed to the fact that KW6002 is a more potent A_{2A} receptor antagonist than CGS15943. Further assessment of the effects of a wider dose range of KW6002 may help to clarify this.

The ability of KW6002 to increase locomotor activity in the Lister hooded rat agrees with the previous observations of KW6002-induced potentiations in rats unilaterally lesioned with 6-OHDA and reduced disability in MPTP-treated non-human primate models (Kanda et al., 1998; Koga et al., 2000; Lundblad et al., 2003). The results are also in general agreement with reported effects of other antagonists such as caffeine (Garrett and Holtzman, 1994; Bedingfield et al., 1998), and the more selective antagonists SCH58261 and KF17837 (Halldner et al., 2000; Correa et al., 2004). It is likely that the locomotor stimulant effects of KW6002 are mediated through actions in the caudate-putamen, rather than NAc, although there is a potential contribution of reward-related exploratory behaviour.

It is of interest to note that KW6002 has shown efficacy in symptom alleviation in pre-clinical and clinical assessments of Parkinson's disease (Bara-Jimenez et al., 2003; Hauser et al., 2003; Lundblad et al., 2003; Chen et al., 2003; Kase et al., 2003; Pierri et al., 2005). Given that the present study has identified that this drug has 'rewarding/reinforcing' effects in an animal model, the potential use of KW6002 as an 'add-on' treatment for Parkinson's disease in conjunction with dopamine mimetics, which themselves may also express reward-related behaviours, deserves further consideration.

Acknowledgements

We thank the BBSRC for the provision of a Research Committee Studentship, Tocris-Cookson for provision of [^{3}H]-ZM241385, Professor Norman Bowery for assistance with monitoring in vitro GABA release and Karen Turner for assistance with the HPLC detection of dopamine.

References

Acquas E, Tanda G, Di Chiara G. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. Neuropsychopharmacology 2002;27:182–93.

Alexander SPH, Millns P. [^{3}H]ZM241385—an antagonist radioligand for adenosine A_{2A} receptors in rat brain. Eur J Pharmacol 2001;411:205–10.

Aoyama S, Kase H, Borrelli E. Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A_{2A} receptor antagonist. J Neurosci 2000;20:5848–52.

Aoyama S, Koga K, Mori A, Miyaji H, Sekine S, Kase H, et al. Distribution of adenosine A_{2A} receptor antagonist KW-6002 and its effect on gene expression in the rat brain. Brain Res 2002;953:119.

Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M, et al. Adenosine A_{2A} receptor antagonist treatment of Parkinson's disease. Neurology 2003;61:293–6.

Bedingfield JB, King DA, Holloway FA. Cocaine and caffeine: conditioned place preference, locomotor activity, and additivity. Pharmacol Biochem Behav 1998;61:291–6.

Cadogan A-K, Alexander SPH, Boyd EA, Kendall DA. Influence of cannabinoids on electrically-evoked dopamine release and cyclic AMP generation in the rat striatum. J Neurochem 1997;69:1131–7.

Cheer JF, Kendall DA, Marsden CA. Cannabinoid receptors and reward in the rat: a conditioned place preference study. Psychopharmacol 2000;151: 25-30.

Chen JF, Moratalla R, Impagnatiello F, Grandy DK, Cuellar B, Rubinstein M, et al. The role of the D2 dopamine receptor (D2R) in A_{2A} adenosine receptor (A_{2A}R)-mediated behavioral and cellular responses as revealed by A_{2A} and D_2 receptor knockout mice. Proc Natl Acad Sci U S A 2001;98:1970–5.

Chen J-F, Fredduzzi S, Bastia E, Yu L, Moratalla R, Ongini E, et al. Adenosine A_{2A} receptors in neuroadaptation to repeated dopaminergic stimulation: implications for the treatment of dyskinesias in Parkinson's disease. Neurology 2003;61:S74–81.

Correa M, Wisniecki A, Betz A, Dobson DR, O'Neill MF, O'Neill MJ, et al. The adenosine A_{2A} antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to parkinsonism. Behav Brain Res 2004;148:47–54.

Corsi C, Pinna A, Gianfriddo M, Melani A, Morelli M, Pedata F. Adenosine A_{2A} receptor antagonism increases striatal glutamate outflow in dopamine-denervated rats. Eur J Pharmacol 2003;464:33–8.

Fenu S, Pinna A, Ongini E, Morelli M. Adenosine A_{2A} receptor antagonism potentiates L-DOPA-induced turning behaviour and c-*fos* expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 1997;321:143–7.

Ferre S, Herrera-Marschitz M, Grabowska-Anden M, Casas M, Ungerstedt U, Anden NE. Postsynaptic dopamine/adenosine interaction. II. Postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. Eur J Pharmacol 1991;192:31–7.

Fulford AJ, Marsden CA. Effect of isolation rearing on conditioned dopamine release in vivo in the nucleus accumbens of the rat. J Neurochem 1998;70:384–90.

Garrett BE, Holtzman SG. D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. Pharmacol Biochem Behav 1994;47:89–94.

Halldner L, Lozza G, Lindstrom K, Fredholm BB. Lack of tolerance to motor stimulant effects of a selective adenosine A_{2A} receptor antagonist. Eur J Pharmacol 2000;406:345–54.

Harper LK, Beckett SRG, Marsden CA, Alexander SPH. A_{2A} adenosine receptor inhibition of dopamine release in the rat nucleus accumbens in vitro. Br J Pharmacol 2002;137(SS) [131 pp.].

Harper LK, Beckett SR, Marsden CA, McCreary AC, Alexander SPH. In vitro and in vivo effects of the A_{2A} adenosine receptor antagonist KW-6002 on rat dopaminergic systems. J Psychopharmacol 2003;17(3):A40.

Harvey V, Jones J, Misra A, Knight AR, Quirk K. Solubilisation and immunoprecipitation of rat striatal adenosine A_{2A} receptors. Eur J Pharmacol 2001;431:171–7.

Hauser RA, Hubble JP, Truong DD. Randomized trial of the adenosine A_{2A} receptor antagonist istradefylline in advanced PD. Neurology 2003; 61:297–303.

Kanda T, Shiozaki S, Shimada J, Suzuki F, Nakamura J. KF17837: a novel selective adenosine A_{2A} receptor antagonist with anticataleptic activity. Eur J Pharmacol 1994;256:263–8.

Kanda T, Tashiro T, Kuwana Y, Jenner P. Adenosine A_{2A} receptors modify motor function in MPTP-treated common marmosets. Neuroreport 1998; 9:2857–60.

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Combined use of the adenosine A_{2A} antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol 2000; 162:321–7.

Karcz-Kubicha M, Quarta D, Hope BT, Antoniou K, Müller CE, Morales M, et al. Enabling role of adenosine A1 receptors in adenosine A_{2A} receptor-mediated striatal expression of c-*fos*. Eur J Neurosci 2003; 18:296–302.

Kase H, Aoyama S, Ichimura M, Ikeda K, Ishii A, Kanda T, et al. Progress in pursuit of therapeutic A_{2A} antagonists: the adenosine A_{2A} receptor selective antagonist KW6002: research and development toward a novel nondopaminergic therapy for Parkinson's disease. Neurology 2003;61: S97–100.

Kirk IP, Richardson PJ. Adenosine A_{2a} receptor-mediated modulation of striatal [^{3}H]GABA and [^{3}H]acetylcholine release. J Neurochem 1994;62:960–6.

Kirk IP, Richardson PJ. Inhibition of striatal GABA release by the adenosine A_{2A} receptor is not mediated by increases in cyclic AMP. J Neurochem 1995;64:2801–9.

Koga K, Kurokawa M, Ochi M, Nakamura J, Kuwana Y. Adenosine A_{2A} receptor antagonists KF17837 and KW-6002 potentiate rotation induced by dopaminergic drugs in hemi-Parkinsonian rats. Eur J Pharmacol 2000; 408:249–55.

Kurokawa M, Kirk IP, Kirkpatrick KA, Kase H, Richardson PJ. Inhibition by KF17837 of adenosine A_{2A} receptor-mediated modulation of striatal GABA and ACh release. Br J Pharmacol 1994;113:43–8.

Kuwana Y, Shiozaki S, Kanda T, Kurokawa M, Koga K, Ochi M, et al. Antiparkinsonian activity of adenosine A_{2A} antagonists in experimental models. Adv Neurol 1999;80:121–3.

Lopes LV, Cunha RA, Ribeiro JA. Increase in the number, G protein coupling, and efficiency of facilitatory adenosine A_{2A} receptors in the limbic cortex, but not striatum, of aged rats. J Neurochem 1999;73:1733–8.

Lundblad M, Vaudano E, Cenci MA. Cellular and behavioural effects of the adenosine A_{2A} receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. J Neurochem 2003;84:1398–410.

Mayfield RD, Suzuki F, Zahniser NR. Adenosine A_{2a} receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. J Neurochem 1993;60:2334–7.

Mayfield RD, Larson G, Orona RA, Zahniser NR. Opposing actions of adenosine A_{2a} and dopamine D_2 receptor activation on GABA release in the basal ganglia: evidence for an A_{2a}/D_2 receptor interaction in globus pallidus. Synapse 1996;22:132–8.

Morelli M, Fenu S, Pinna A, Di Chiara G. Adenosine A2 receptors interact negatively with dopamine D1 and D2 receptors in unilaterally 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 1994;251:21–5.

Ochi M, Koga K, Kurokawa M, Kase H, Nakamura J, Kuwana Y. Systemic administration of adenosine A_{2A} receptor antagonist reverses increased GABA release in the globus pallidus of unilateral 6-hydroxydopamine-lesioned rats: a microdialysis study. Neuroscience 2000; 100:53–62.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego, CA: Academic Press; 1997.

Pierri M, Vaudan E, Sager T, Englund U. KW-6002 protects from MPTP induced dopaminergic toxicity in the mouse. Neuropharmacol 2005;48: 517-524.

Pollack AE, Fink JS. Synergistic interaction between an adenosine antagonist and a D1 dopamine agonist on rotational behavior and striatal c-*fos* induction in 6-hydroxydopamine-lesioned rats. Brain Res 1996;743: 124-130.

Popoli P, Betto P, Reggio R, Ricciarello G. Adenosine A_{2A} receptor stimulation enhances striatal extracellular glutamate levels in rats. Eur J Pharmacol 1995;287:215–7.

Richardson PJ, Kase H, Jenner PG. Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci 1997;18:338–44.

Shimada J, Koike N, Nonaka H, Shiozaki S, Yanagawa K, Kanda T, et al. Adenosine A_{2A} antagonists with potent anti-cataleptic activity. Bioorg Med Chem Lett 1997;7:2349–52.

Shiozaki S, Ichikawa S, Nakamura J, Kitamura S, Yamada K, Kuwana Y. Actions of adenosine A_{2A} receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacol 1999;147:90–5.

Solinas M, Ferre S, You ZB, Karcz-Kubicha M, Popoli P, Goldberg SR. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J Neurosci 2002;22:6321–4.

Zocchi C, Ongini E, Conti A, Monopoli A, Negretti A, Baraldi PG, et al. The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A_{2a} adenosine receptor antagonist. J Pharmacol Exp Ther 1996; 276:398–404.