Activation of Adenosine₁ Receptors Induces Antidepressant-Like, Anti-Impulsive Effects on Differential Reinforcement of Low-Rate 72-s Behavior in Rats

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ABSTRACT

Stress and psychiatric illness have been associated with a dysregulation of glutamatergic neurotransmission. Recently, positive allosteric modulators (PAMs) of the metabotropic glutamate 2 (mGlu₂) receptor have been found to exert antidepressant-like activity in rats performing under a differential reinforcement of low rate (DRL) 72-s schedule. An autoreceptor role at glutamatergic synapses is the most salient physiological role played by the mGlu₂ receptor. Adenosine A₁ receptors play a heteroreceptor role at many of the same forebrain synapses where mGlu₂ autoreceptors are found. Agonists and/or PAMs of mGlu₂ receptors act similarly to adenosine A₁ receptor agonists with respect to a wide range of electrophysiological, biochemical, and behavioral responses mediated by limbic circuitry thought to play a role in the pathophysiology of neuropsychiatric disease and to mediate therapeutic drug effects. Therefore, the role of adenosine A₁ receptor activation on rat DRL 72-s behavior was explored to provide preclinical evidence consistent or inconsistent with potential antidepressant effects. The adenosine A₁ receptor agonist N^6-cyclohexyladenosine (CHA) increased the reinforcement rate, decreased the response rate, and induced a rightward shift in inter-response time distributions in a dose-dependent fashion similar to most known antidepressant drugs. The adenosine A₁ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) blocked these antidepressant-like effects. These novel observations with CHA and DPCPX suggest that activation of adenosine A₁ receptors could contribute to antidepressant effects, in addition to previous preclinical reports of anxiolytic and antipsychotic effects. By implication, targeting a dysregulated glutamatergic system may be an important principle in discovering novel antidepressant agents that may also possess anti-impulsive activity.

Introduction

Stress is known to play a major role in psychiatric illness. Major depression and bipolar depression are associated with increased or decreased blood flow in the medial orbital prefrontal cortex (PFC) and a number of other limbic-related brain regions, suggesting dysregulation of glutamatergic neurotransmission. Furthermore, antidepressant treatments normalize increased cortical blood flow (Price and Drevets, 2010). Metabotropic glutamate 2/3 (mGlu₂/₃) receptor agonism is an effective therapeutic approach for neuropsychiatric illness with divergent etiology/pathophysiology such as generalized anxiety disorder and schizophrenia (Patil et al.,

2007; Dunayevich et al., 2008), disorders where stress and/or dysregulated glutamate release at key forebrain regions plays a key pathophysiological role. Activation of mGlu₂ receptors plays a role in preclinical anxiety and psychosis "efficacy" models extrapolating from experiments with transgenic mice lacking mGlu₂ and/or mGlu₃ receptors or from comparisons of mGlu₂ receptor positive allosteric modulators (PAMs) with orthosteric mGlu₂/₃ receptor agonists. An glutamatergic autoreceptor function is the most prominent physiological role played by mGlu₂ receptors; mGlu₂ receptor activation suppresses glutamate release at many synapses throughout the limbic forebrain (Schoepp, 2001). Preclinical studies with mGlu₂ receptor PAMs have suggested that this novel class of drugs may possess antidepressant properties (Nikiforuk et al., 2010; Fell et al., 2011).

Although activation of mGlu₂ receptors may play a therapeutic role in the treatment of schizophrenia and generalized anxiety disorder, preclinical evidence suggests that activation of mGlu₂ and/or mGlu₃ receptors could play a role in antinoci-

ABBREVIATIONS: PFC, prefrontal cortex; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DRL, differential reinforcement of low rate; FST, forced swim test; mGlu, metabotropic glutamate; CHA, N^6-cyclohexyladenosine; PAM, positive allosteric modulator; IRT, inter-response time; MK-801, $(5S,10R)$-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; 5-HT, 5-hydroxytryptamine; K-S, Kolmogorov-Smirnov.

ptive effects (including antimigraine effects), attenuation of opioid withdrawal effects, and general neuroprotective and antiepileptic effects. Preclinical evidence suggests that the activation of adenosine A$_1$ receptors plays a similar role for this wide range of neuropsychiatric indications (Ribeiro et al., 2002; Kaster et al., 2004; Marek, 2009). The similar distribution of mGlu$_2$ and adenosine A$_1$ receptors throughout the limbic forebrain probably is an important underpinning to similar effects of activating these receptors across a wide range of neuropsychiatric preclinical models (Bauer et al., 2003; Richards et al., 2005). Hallucinogen-induced head shakes and hallucinogen/(5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801)-induced immediate early gene expression are modulated by mGlu$_2$ or adenosine A$_1$ receptors in the PFC or on thalamocortical pathways (Scruggs et al., 2000; Gotoh et al., 2002; Pei et al., 2004; Benneyworth et al., 2007; Marek, 2009; Wischhof and Koch, 2012). Although adenosine A$_1$ receptors are located at both postsynaptic and presynaptic sites, these receptors are known to play a prominent role as heteroreceptors, decreasing excitatory amino acid release, similar to the mGlu$_2$ receptor role as an autoreceptor.

Although mGlu$_{2/3}$ receptor agonist prodrugs have been demonstrated in double-blind, placebo-controlled trials to exert antipsychotic or anxiolytic action, adenosine A$_1$ receptor agonists act similarly to many of the mGlu$_{2/3}$ receptor agonists in a wide variety of the same preclinical screens detecting antipsychotic (reviewed by Marek, 2009) or anxiolytic drugs. Adenosine A$_1$ receptor agonists possess antidepressant-like tests in mice in the forced swim test (FST) and the tail suspension test (Kaster et al., 2004). An additional reason for testing potential antidepressant activity of adenosine A$_1$ receptor agonists is that these compounds presynaptically suppress glutamate release induced by 5-HT$_{2A}$ receptor activation in the PFC, similarly to activation of mGlu$_2$ autoreceptors (Marek et al., 2000; Stutzmann et al., 2001). The DRL 72-s schedule is an antidepressant screen where 5-HT$_{2A}$ receptor antagonists exert frank antidepressant effects and also enhance the antidepressant-like effects of tricyclic antidepressants, selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors (Marek et al., 2005; Ardayfio et al., 2008). From a clinical standpoint, blockade of 5-HT$_{2A}$ receptors may contribute to the antidepressant effects of tricyclic antidepressants, heterocyclic antidepressants (e.g., mianserin, mirtazapine, trazodone, and nefazodone), and atypical antipsychotics (e.g., quetiapine, risperidone, olanzapine, and aripiprazole).

Because the activation of mGlu$_2$ receptors (Nikiforuk et al., 2010; Fell et al., 2011) and the blockade of 5-HT$_{2A}$ receptors results in antidepressant activity in rats performing under a DRL 72-s schedule potentially by attenuating motoric impulsivity, the effects of a selective adenosine A$_1$ receptor agonist, N^6-cyclohexyladenosine (CHA), both alone and with a selective adenosine A$_1$ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX or PD116,948), were tested in rats trained to perform on a DRL 72-s schedule. The results with CHA are consistent with previous literature suggesting antidepressant-like activity with the activation of adenosine A$_1$ receptors (Kaster et al., 2004). The present results on DRL 72-s behavior also are consistent with the working hypothesis that the mechanism underlying these antidepressant-like effects involves an attenuation of motoric impulsivity. These results add to a growing literature suggesting that modula-

tion of glutamatergic activity may lead to the development of truly novel antidepressant and/or mood-stabilizing drugs.

Materials and Methods

Animals. For the DRL experiments, 21 male Sprague-Dawley rats weighing between 300 and 350 g at the beginning of the behavioral experiments (Holtzman, Madison, WI) were housed in suspended stainless-steel wire cages (18 × 36 × 20 cm) with two rats occupying each cage. The colony room was maintained at 20°C and relative humidity (60%). The room was illuminated 12 h/day (7:00 AM–7:00 PM). All rats had free access to laboratory chow (Teklad 4% rat diet; Harlan Laboratories, Madison, WI) except during experimental sessions. Water was available for only a 20-min period after the daily behavioral session except for Friday after the DRL session when water was available ad libitum until approximately noon Sunday. All animals were treated in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996), and all protocols were approved by the Yale University Animal Care and Use Committee.

Apparatus. Eight operant-conditioning chambers (30.5 × 24.1 × 29.2 cm; MED Associates, St. Albans, VT) were used for the DRL experiments. The levers in these chambers were mounted on one wall with the water access next to the levers in the middle of the wall. A reinforced response caused the dipper (0.02-cc cup) to be lifted from a water trough to an opening in the floor of the access port for 4 s. The house light (which was mounted on the opposite wall) was turned on when the session began, remained on throughout the entire session, and was turned off at the end of the session. Each experimental chamber was enclosed in a melamine sound-attenuating cubicle and equipped with a white-noise generator to provide masking noise.

Operant Training. Rats were water-deprived for ~22.5 h before each session. Each rat was initially trained under an alternating fixed-ratio 1, fixed-time 1-min schedule for water reinforcement. Thus, each response was reinforced, and water was also provided every minute if a response did not occur. The few rats that did not acquire lever-pressing behavior after three daily 1-h sessions under this schedule were trained by the experimenter using the method of successive approximation. After the rats had acquired lever-pressing behavior they were trained during daily DRL 18-s sessions for ~2 weeks before moving directly to DRL 72-s sessions. The responding on these sessions became stable after ~8 weeks. Experimental sessions lasted for 1 h and were conducted 5 days/week during light hours.

Schedule Control and Statistical Analyses. The experimental chambers were connected to a computer via a MED-SYS-8 interface and software package (Med Associates). All behavioral data are expressed as the mean ± S.E.M., in some cases normalized to the vehicle or vehicle–vehicle condition. The DRL data analysis was performed on nontransformed data. The effects of CHA (15.6–125 μg/kg) and DPCPX (0.313 mg/kg) alone were analyzed with a one-factor repeated-measures analysis of variance. The effects of the combined treatment of CHA and DPCPX were analyzed by using a two-factor repeated-measures analysis of variance with CHA and DPCPX as within-subject factors. Significant main effects or interactions were analyzed by the Newman-Keuls or Dunnett test where appropriate. The level of significance was set for $p < 0.05$ for all analyses. The cumulative inter-response time (IRT) distribution for the entire group of rats treated with a within-subject design were analyzed with the nonparametric Kolmogorov-Smirnov (K-S) test to make comparisons between different treatment conditions. The level of significance was set for $p < 0.05$.

Drugs. Doses were calculated on the basis of the salt forms. The drugs were dissolved in saline, neutralized to a pH ~7.4, and injected by using a volume of 1 ml/kg body weight. The adenosine A$_1$ receptor agonist CHA and the adenosine A$_1$ receptor antagonist DPCPX were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris Bioscience
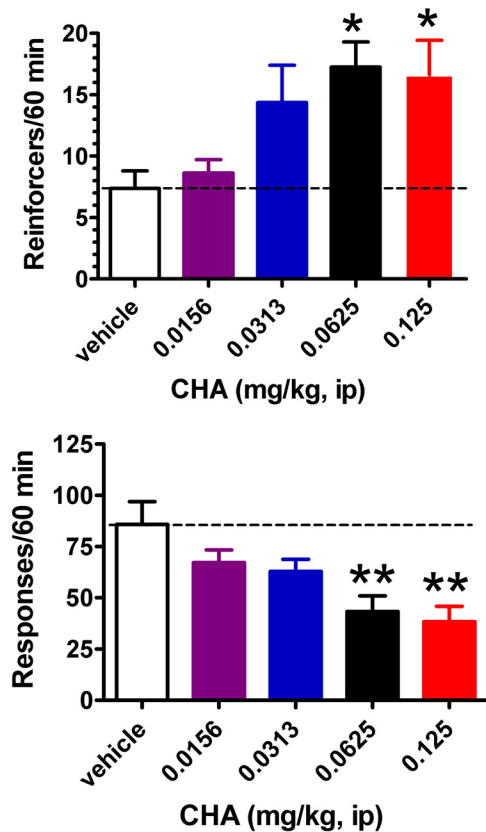
Fig. 1. Dose-dependent changes in the frequency of reinforcers and responses induced by the adenosine A_1 receptor agonist CHA (0.0156–0.125 mg/kg i.p.) for rats performing under a DRL 72-s schedule ($n = 8$). For the vehicle condition, the mean ($\pm$ S.E.M.) number of reinforcers obtained was 7.9 ($\pm$ 1.49) while the mean number of total responses was 86.8 ($\pm$ 11.20). Rats were injected 1 h before a 1-h behavioral session. Significant changes from the vehicle control are indicated: *, $p < 0.05$, and **, $p < 0.01$, using the Dunnett test. Dotted lines indicate graphical continuation of the vehicle control value.

(Ellisville, MO), respectively. Drugs were administered to the animals only once weekly by an intraperitoneal route of administration 1 h before the behavioral session (Thursday). All drug treatments were carried out only once weekly to minimize possible carryover effects.

Results

The behavior of each rat seemed to return to baseline the day after drug administration.

Antidepressant-Like Actions of CHA on DRL 72-s Behavior. The adenosine A_1 receptor agonist CHA (0.0156–0.125 mg/kg i.p.) increased the number of reinforcers earned for rats performing under a DRL 72-s operant schedule in a dose-dependent manner ($F_{4,28} = 3.91$; $p < 0.05$; Fig. 1). Post hoc testing revealed significant increases at the 0.0625 and 0.125 mg/kg dose conditions (Dunnett t test; $p < 0.05$) compared with the vehicle condition. CHA also decreased the total lever press responses in a dose-dependent manner ($F_{4,28} = 8.27$; $p < 0.001$) with significant decreases at the 0.0625 and 0.125 mg/kg dose conditions (Dunnett t test; $p < 0.01$).

An apparent dose-dependent rightward shift in the IRT distributions was also observed after CHA administration (Fig. 2). This change was suggested by a significantly different IRT distribution for the 0.0625 and 0.125 mg/kg CHA
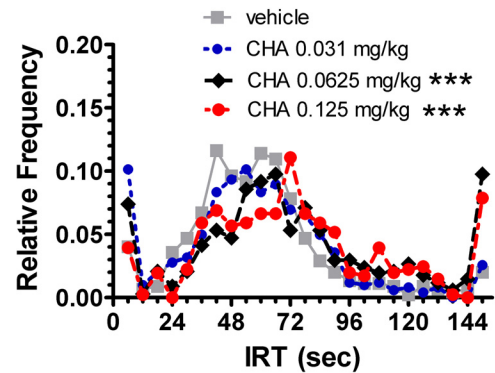

Fig. 2. Effects of the adenosine A_1 receptor agonist CHA (0.0313–0.125 mg/kg) on inter-response time (IRT) distributions for rats performing on a DRL 72-s schedule. Cumulative IRT for this group of rats obtaining the relative frequency of all responses for each rat separately for each dose condition. The cumulative IRT distribution for the entire group for each CHA dose was compared with the group cumulative IRT distribution for the vehicle condition by using the nonparametric Kolmogorov-Smirnov test. ***, $p < 0.001$.

doses compared with the vehicle condition when examining the cumulative IRT distributions for the entire group of eight rats ($p < 0.001$; K-S test). The changes in the temporal pattern of responding observed for the cumulative IRT distributions of the entire group were also seen when examining IRT distributions for the individual rats. Significantly different IRT distributions from the vehicle condition were observed for zero, four, and six of eight rats at the 0.0156, 0.0313, and 0.0625 mg/kg CHA doses, respectively ($p < 0.001$; K-S test). One rat at the 0.125 mg/kg CHA dose did not respond; five of the remaining seven rats had IRT distributions significantly different from the vehicle condition ($p < 0.001$). A numerical trend for a rightward shift in the mean IRT value approaching the 72-s schedule time requirement occurred after administration of the adenosine A_1 receptor agonist CHA [mean IRTs (S.D.): vehicle, 57.5 (42.6) s; 0.0313 mg/kg, 56.0 (40.2) s; 0.0625 mg/kg, 81.9 (93.8) s; 0.125 mg/kg, 77.6 (62.8) s].

Effects of the Adenosine A_1 Receptor Antagonist DPCPX on DRL 72-s Behavior. The adenosine A_1 receptor antagonist DPCPX (0.31 mg/kg i.p.) alone did not alter the frequency of reinforcers obtained for rats responding on a DRL 72-s schedule compared with vehicle ($t_{12} = 1.46$; $p > 0.1$; Fig. 3) in a second cohort of 13 rats. However, DPCPX (0.31 mg/kg) did increase the frequency of responses compared with vehicle ($t_{12} = 3.64$; $p < 0.01$). The cumulative IRT distribution for the entire group of rats was not altered for the DPCPX treatment compared with the vehicle condition ($p > 0.1$; K-S test; data not shown). Likewise, the mean ($\pm$ S.D.) IRT interval was unchanged for the DPCPX treatment (38.7 $\pm$ 26.2 s) compared with vehicle (37.6 $\pm$ 26.1 s). In a subset ($n = 7$) of this cohort an extended DPCPX dose range was tested (2.5, 5, and 10 mg/kg versus placebo). There was no effect of DPCPX on the number of reinforcers obtained ($F_{3,18} = 2.22$; $p = 0.121$), although there was a trend for an increased number in the total number of lever presses ($F_{3,18} = 2.74$; $p = 0.074$).

DPCPX Blocks the Antidepressant-Like Effects of CHA on DRL 72-s Behavior. In a third experiment using the second cohort of rats, CHA (0.0625 mg/kg i.p.) increased the frequency of reinforcers obtained for rats performing under a DRL 72-s schedule, and this effect of CHA was
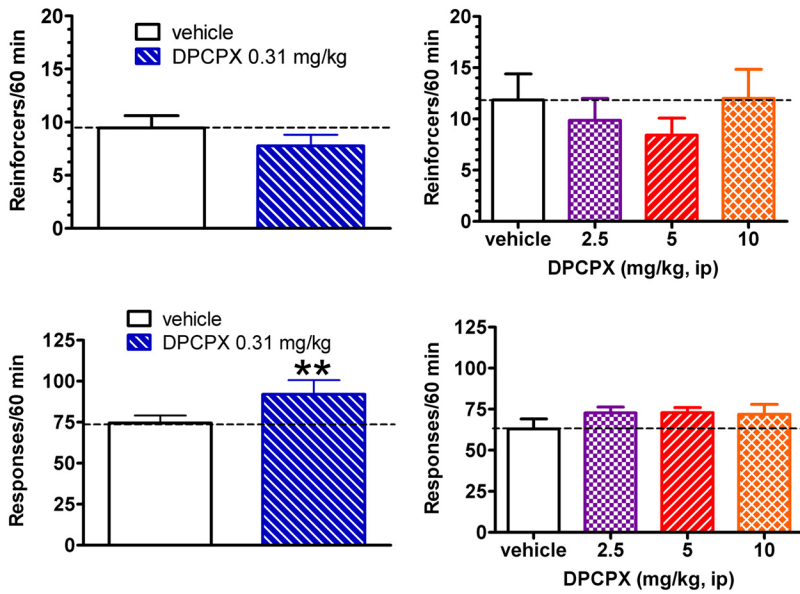
Fig. 3. Effects of the adenosine A$_1$ receptor antagonist DPCPX (0.31 mg/kg i.p., n = 13, graphs on left; 2.5–10 mg/kg, n = 7, graphs on right) on the number of reinforcers obtained (top) and the total responses (bottom) made by rats performing on a DRL 72-s schedule. Open bars, vehicle; hatched bars, DPCPX. **, $p < 0.01$ compared with the vehicle condition.

blocked by the adenosine A$_1$ receptor antagonist DPCPX ($F_{4,48}$ = 11.58; $p < 0.0001$; Fig. 4). CHA significantly increased the reinforcement rate by ~100% compared with the vehicle–vehicle condition both times the adenosine receptor agonist was administered ($p < 0.001$; Newman-Keuls test; second vehicle/CHA treatment not shown). In contrast, the reinforcement rate was unchanged compared with the vehicle–vehicle condition when DPCPX was coadministered with CHA at either the 0.31, 1.25, or 5 mg/kg dose. However, the number of reinforcers obtained for each dose of DPCPX administered with CHA was significantly decreased compared with the vehicle–CHA condition ($p < 0.001$; 0.31, 1.25, or 5 mg/kg DPCPX administered with CHA; Newman-Keuls test).

CHA (0.0625 mg/kg i.p.) decreased the total response frequency, and this CHA effect was blocked by the adenosine A$_1$ receptor antagonist DPCPX ($F_{4,48}$ = 11.15; $p < 0.0001$; Fig. 4). CHA significantly decreased the response rate by 24 to 37% compared with the vehicle–vehicle condition both times the adenosine receptor agonist was administered [$p < 0.001$ and < 0.01; (second administration not shown), respectively, Newman-Keuls test]. In contrast, the response rate was unchanged from the vehicle–vehicle condition when DPCPX was coadministered with CHA. However, the total number of lever press responses for each dose of DPCPX administered with CHA was significantly increased compared with the vehicle–CHA condition ($p < 0.001$; Newman-Keuls test).

The rightward shift of the IRT distribution induced by CHA (0.0625 mg/kg) was blocked by the adenosine A$_1$ receptor antagonist DPCPX (0.31 mg/kg) (Fig. 5). CHA induced a significant alteration in the cumulative group IRT distribution (n = 13; $p < 0.001$; K-S test; mean ± S.D. of the IRTs, 52.3 ± 38.5 s) compared with the vehicle condition (mean IRT ± S.D., 37.7 ± 26.1 s). In contrast, the IRT distribution was not significantly altered by administration of both CHA and DPCPX (mean IRT ± S.D., 35.7 ± 26.0 s) compared with the vehicle condition. Rechallenge with CHA (0.0625 mg/kg) again resulted in a significant shift in the cumulative group IRT distribution ($p < 0.001$; K-S test; mean IRT ± S.D., 47.7 ± 47.0 s) compared with the vehicle–vehicle condition.

Discussion

This is the first known demonstration that the activation of adenosine A$_1$ receptors, potentially by suppressing glutamate release in the medial PFC and associated limbic macrocircuitry, results in effects similar to antidepressant drugs by using the rat DRL 72-s schedule of reinforcement. The known pharmacological selectivity of both CHA and DPCPX for adenosine A$_1$ receptors indicates that the effects of these drugs are mediated via activation and blockade of adenosine A$_1$ receptors. Although CHA is only modestly selective for rat adenosine A$_1$ compared with rat adenosine A$_3$ receptors (>200-fold), this agonist is nearly 500-fold and more than 10,000-fold selective for rat A$_{2A}$ and human A$_{2B}$ receptors (Bruns, 1980; Daly et al., 1993; van Galen et al., 1994) Although DPCPX is only modestly selective for adenosine A$_1$ compared with A$_{2B}$ receptors (~400-fold), this antagonist is nearly 300- to 1000-fold and 10,000-fold selective compared with rodent A$_{2A}$ and A$_3$ receptors, respectively (Bruns, 1980; Auchampach et al., 1997; Weyler et al., 2006). Thus, the potent antagonism exerted by DPCPX against CHA is mediated by their most potent shared site of action, adenosine A$_1$ receptors.

Furthermore, previous in vivo experiments suggest that the doses of CHA and DPCPX chosen for the present experiments do specifically interact with adenosine A$_1$ receptors. First, complete displacement of the binding for a different adenosine A$_1$ receptor radiotracer was obtained with a 1 mg/kg i.p. dose of DPCPX (Paul et al., 2011). Second, CHA and DPCPX (each 0.03–0.30 mg/kg doses) altered locomotor activity through a specific interaction with brain adenosine A$_1$ receptors (Marston et al., 1998). Third, although adenosine A$_1$ and adenosine A$_{2A}$ receptors easily are the most abundant adenosine receptor subtypes found in the brain (Ribeiro et al., 2002), DPCPX (0.375–1.5 mg/kg i.p.) failed to attenuate the locomotor effects of the dopamine D$_2$ receptor antagonist eticlopride unlike an adenosine A$_{2A}$ receptor antagonist (Collins et al., 2010). Unfortunately, the 0.3 mg/kg DPCPX dose completely suppressed the antidepressant-like effect of CHA. A more complete understanding of CHA and DPCPX pharmacological potency that was available after the
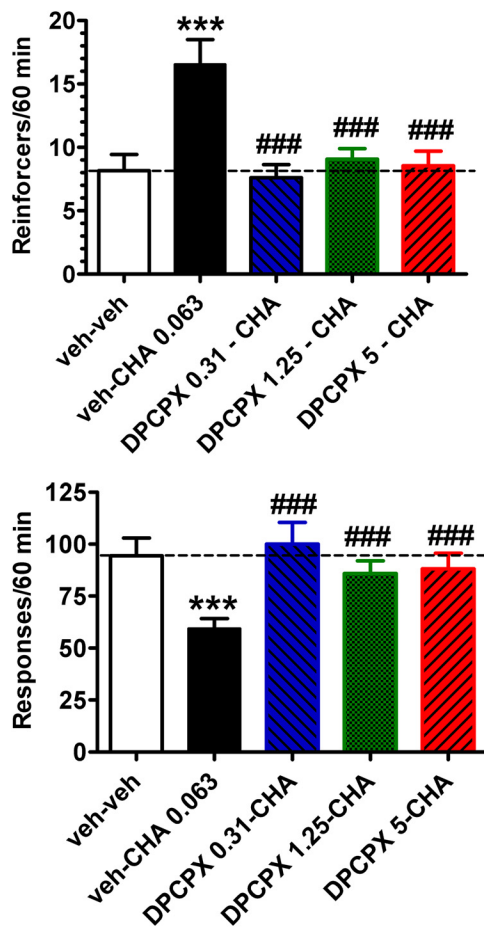
Fig. 5. Effects of the adenosine A_1 receptor agonist CHA (0.0625 mg/kg) on IRT distributions for rats performing on a DRL 72-s schedule in both the absence and the presence of the adenosine A_1 receptor antagonist DPCPX (0.31 mg/kg). Cumulative IRT distributions for the entire group of 13 rats are plotted for each of the respective dose conditions, including a replication of the CHA-alone effect. Only the two different CHA-alone treatments were significantly different from the vehicle condition. ∗∗∗, $p < 0.001$, K-S test.

Fig. 4. Effects of the adenosine A_1 receptor antagonist DPCPX (0.31, 1.25, and 5 mg/kg) on the antidepressant-like effects of the adenosine A_1 receptor agonist CHA (0.0625 mg/kg) in rats performing on a DRL 72-s schedule. Rats were tested using a with-in subject design with drugs administered at weekly intervals in the order shown ($n = 13$) via an intraperitoneal route of administration. Open bar, vehicle–vehicle; solid black bars, vehicle–CHA (0.0625 mg/kg); blue hatched bars, DPCPX (0.31 mg/kg)–CHA (0.0625 mg/kg).; green cross-hatched bars, DPCPX (1.25 mg/kg)–CHA (0.0625 mg/kg); red hatched bars, DPCPX (5 mg/kg)–CHA (0.0625 mg/kg). ∗∗∗, $p < 0.001$ compared with the vehicle–vehicle condition. ###, $p \leq 0.001$ compared with the vehicle–CHA (0.0625 mg/kg) condition. The significant effects of the combination of vehicle–CHA (0.0625 mg/kg) administered a second time compared with the vehicle–vehicle condition are not shown.

present studies (Collins et al., 2010; Paul et al., 2011) were conducted suggests that the 0.3 mg/kg DPCPX dose results in maximal effects at blocking adenosine A_1 receptors. Furthermore, similar antidepressant-like effects of CHA alone were observed in this experiment both times the agonist was administered. Phosphodiesterase-4 inhibition also results in antidepressant-like effects on DRL behavior that may be mediated by increases in cAMP (reviewed in O'Donnell et al., 2005), raising the question as to whether adenosine A_1 receptors with transduction pathways through G_s proteins and increased cAMP (adenosine A_{2A} or A_{2B} receptors) might play a role in the effects of CHA and DPCPX. However, the common in vitro and in vivo pharmacological potency of both CHA and DPCPX only for adenosine A_1 receptors suggests that this adenosine receptor subtype mediates the antidepressant-like action of CHA on DRL behavior.

The prominent presynaptic localization of adenosine A_1 receptors throughout the limbic forebrain is consistent with
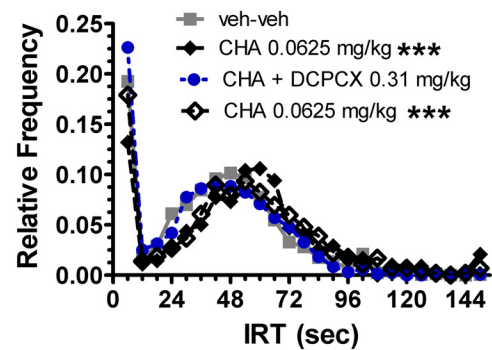
the hypothesis that the suppression of glutamate release in forebrain circuits may be responsible for the antidepressant-like effects of CHA on DRL behavior. Synapses where adenosine A_1 receptors suppress glutamate release include thalamocortical (Fontanez and Porter, 2006), thalamostriatal (Flagmeyer et al., 1997), corticostriatal (Flagmeyer et al., 1997), the hippocampal formation including the perforant pathway (Dragunow et al., 1988), and the subthalamic nucleus (Shen and Johnson, 2003). Adenosine A_1 receptors also seem to modulate corpus callosal axon physiology (Swanson et al., 1998). However, these considerations do not rule out a role for postsynaptic adenosine A_1 receptors.

A significant overlap exists between forebrain distributions of $mGlu_2$ and adenosine A_1 receptors in thalamocortical pathways (regulated by $5-HT_{2A}$ receptor activation) from midline/intralaminar thalamic nuclei (Marek et al., 2001; Stutzmann et al., 2001; Benneyworth et al., 2007), the medial perforant pathways in the hippocampal formation (Dragunow et al., 1988; Shigemoto et al., 1997), and corticostriatal pathways (Flagmeyer et al., 1997; Conn et al., 2005; Johnson et al., 2005) where both receptors play autoreceptor or heteroreceptor functions. Given the prominent autoreceptor and heteroreceptor effects of $mGlu_2$ and adenosine A_1 receptors in the central nervous system it is parsimonious to propose the working hypothesis that the antidepressant-like effects observed with $mGlu_2$ receptor PAMs (Nikiforuk et al., 2010; Fell et al., 2011) and the adenosine A_1 receptor agonist CHA on DRL 72-s behavior is attributable to a suppression of glutamate release at limbic synapses.

Regarding a triangulation of $5-HT_{2A}$, $mGlu_2$, and adenosine A_1 receptors on operant behavior, the highly selective $5-HT_{2A}$ receptor antagonist M100907 exerts effects similar to most antidepressant drugs with DRL 72-s behavior (Marek et al., 2005; Ardayfio et al., 2008). The present demonstration that CHA exerted effects on DRL 72-s behavior similar to most antidepressant drugs is interesting given the previously documented antidepressant-like effects for several $mGlu_2$ receptor PAMs (Nikiforuk et al., 2010; Fell et al., 2011). Thus, the hypothesis that both $mGlu_2$ receptor PAMs and adenosine A_1 receptor agonists induce antidepressant-like effects on DRL 72-s behavior by virtue of attenuating glutamate release in limbic synapses is driven by 1) overlapping ana-

tomical distribution of mGlu$_2$ and adenosine A$_1$ receptors, 2) activation of both receptors suppressing glutamate release, 3) activation of both receptors suppressing excitatory synaptic currents induced by 5-HT$_{2A}$ receptor activation, and 4) mGlu$_2$ receptor activation suppressing hallucinogen-induced immediate early gene expression in the medial PFC.

How might CHA, mGlu$_2$ receptor PAMs, and selective 5-HT$_{2A}$ receptor antagonists exert antidepressant-like effects on DRL behavior? Alteration of sedation is one potential explanation for the effects of CHA and DPCPX on DRL 72-s behavior. Although this could contribute to the behavioral effects of CHA, numerous drugs that decrease the response rate and are known to have sedative action in both animals and humans such as typical antipsychotic drugs, alcohol, antihistamines, α_1-adrenergic receptor antagonists, 5-HT$_{1B/1C}$ receptor agonists, and the peripheral administration of 5-HT generally do not induce an antidepressant-like effect on DRL 72-s as reviewed elsewhere (O'Donnell et al., 2005).

Thus, a different explanation is required to understand how 5-HT$_{2A}$ receptor antagonists, CHA, and mGlu$_2$ receptor PAMs induce a constellation of effects similar to most antidepressant drugs on DRL behavior independent of simply a sedative-like decrease in the response rate. Alterations of motoric impulsivity (behavioral inhibition) may be a shared explanatory construct (Pattij et al., 2003; Navarra et al., 2008; Robinson et al., 2008) for these drugs and a range of other drugs including tricyclic antidepressants and norepinephrine transporter inhibitors (Winstanley et al., 2004; Greco et al., 2005; Carli et al., 2006; Blondeau and Dellu-Hagedorn, 2007; Paine et al., 2007; Wischhof and Koch, 2012). This potential involvement of impulsivity in mediating the antidepressant-like effects of norepinephrine transporter inhibitors, tricyclic antidepressants, 5-HT$_{2A}$ receptor antagonists, mGlu$_2$ receptor PAMs, and adenosine A$_1$ receptor agonists on DRL behavior highlights an important differential feature compared with the FST, for which adenosine A$_1$ receptor agonists and mGlu2 receptor agonists also test similarly to antidepressants. Rather than potentially modulating impulsivity on DRL behavior, the FST measures the ability of drugs to modulate an escape behavior. Learned helplessness provides a well known theoretical background for the FST.

Caveats regarding the present study include 1) the use of a single adenosine A$_1$ receptor agonist and antagonist to examine DRL behavior and 2) the failure to demonstrate a no-effect dose for the adenosine A$_1$ receptor antagonist in blocking the effects of CHA. However, the novel antidepressant-like action reported here for CHA agrees with an earlier study suggesting that activation of adenosine A$_1$ receptors mediates antidepressant-like effects on the FST and the tail suspension test (Kaster et al., 2004). The convergence of these different antidepressant screens is intriguing because they would seem to measure different behavioral constructs. Adenosine A$_1$ receptor agonists also decrease rapid eye movement sleep, consistent with the preclinical/clinical action of most antidepressants (Schwierin et al., 1996). This confluence of positive results on DRL 72-s behavior, both the FST and tail suspension test, and suppression of rapid eye movement sleep increases the probability of these divergent behavioral screens correctly predicting effects in patients. Adenosine A$_1$ receptor agonists are not available to test the general hypothesis that decreasing glutamate release in lim-

bic circuitry will result in antidepressant effects in depressed patients because of the peripheral on-target cardiovascular toxicity. However, administration of mGlu$_2$ receptor PAMs could test this hypothesis. Although one mGlu$_2$ receptor PAM has been tested for antipsychotic effects by AstraZeneca Pharmaceuticals LP (Wilmington, DE) and another Johnson & Johnson (New Brunswick, NJ) phase 2 schizophrenia study is currently recruiting subjects to test JNJ-40411813 (www.clinicaltrials.gov), testing of these novel glutamatergic drugs in depressed patients should be pursued (Fell et al., 2011).

Authorship Contributions

Participated in research design: Marek.
Conducted experiments: Marek.
Performed data analysis: Marek.
Wrote or contributed to the writing of the manuscript: Marek.

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