Activation of Adenosine$_1$ 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 (mGlu$_2$) 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 mGlu$_2$ receptor. Adenosine A$_1$ receptors play a heteroreceptor role at many of the same forebrain synapses where mGlu$_2$ autoreceptors are found. Agonists and/or PAMs of mGlu$_2$ receptors act similarly to adenosine A$_1$ 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 A$_1$ receptor activation on rat DRL 72-s behavior was explored to provide preclinical evidence consistent or inconsistent with potential antidepressant effects. The adenosine A$_1$ receptor agonist $N^\circ$-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 A$_1$ 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 A$_1$ 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 (mGlu$_2$/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 mGlu$_2$ receptors plays a role in preclinical anxiety and psychosis “efficacy” models extrapolating from experiments with transgenic mice lacking mGlu$_2$ and/or mGlu$_3$ receptors or from comparisons of mGlu$_2$ receptor positive allosteric modulators (PAMs) with orthosteric mGlu$_2/3$ receptor agonists. An glutamatergic autoreceptor function is the most prominent physiological role played by mGlu$_2$ receptors; mGlu$_2$ receptor activation suppresses glutamate release at many synapses throughout the limbic forebrain (Schoepp, 2001). Preclinical studies with mGlu$_2$ 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 mGlu$_2$ receptors may play a therapeutic role in the treatment of schizophrenia and generalized anxiety disorder, preclinical evidence suggests that activation of mGlu$_2$ and/or mGlu$_3$ receptors could play a role in antinocice-
Adenosine A<sub>1</sub> Receptor Ligands and DRL Behavior

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-cu 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 were 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<sub>1</sub> receptor agonist CHA and the adenosine A<sub>1</sub> receptor antagonist DPCPX were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris Bioscience.
Significant changes from the vehicle control are indicated:

For the vehicle condition, the mean (± S.E.M.) number of reinforcers obtained was 7.9 (± 1.49) while the mean number of total responses was 86.8 (± 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.

Fig. 1. Dose-dependent changes in the frequency of reinforcers and responses induced by the adenosine A<sub>1</sub> 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 (± S.E.M.) number of reinforcers obtained was 7.9 (± 1.49) while the mean number of total responses was 86.8 (± 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.

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<sub>1</sub> 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<sub>4.28</sub> = 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<sub>4.28</sub> = 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 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<sub>1</sub> 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<sub>1</sub> Receptor Antagonist DPCPX on DRL 72-s Behavior. The adenosine A<sub>1</sub> 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<sub>12</sub> = 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<sub>12</sub> = 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 (± S.D.) IRT interval was unchanged for the DPCPX treatment (38.7 ± 26.2 s) compared with vehicle (37.6 ± 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<sub>3.18</sub> = 2.22; p = 0.121), although there was a trend for an increased number in the total number of lever presses (F<sub>3.18</sub> = 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...
The agonist was administered (vehicle–vehicle condition both times the adenosine receptor increased the reinforcement rate by 100% compared with the vehicle–CHA condition (administered with CHA was significantly decreased 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 A1 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; 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).

The rightward shift of the IRT distribution induced by CHA (0.0625 mg/kg) was blocked by the adenosine A1 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 A1 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 A1 receptors indicates that the effects of these drugs are mediated via activation and blockade of adenosine A1 receptors. Although CHA is only modestly selective for rat adenosine A1 compared with rat adenosine A3 receptors (about 200-fold), this agonist is nearly 500-fold and more than 10,000-fold selective for rat A2A and human A2B receptors (Bruns, 1980; Daly et al., 1993; van Galen et al., 1994) Although DPCPX is only modestly selective for adenosine A1 compared with A2B receptors (~400-fold), this antagonist is nearly 300- to 1000-fold and 10,000-fold selective compared with rodent A2A and A3 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 A1 receptors.

Furthermore, previous in vivo experiments suggest that the doses of CHA and DPCPX chosen for the present experiments do specifically interact with adenosine A1 receptors. First, complete displacement of the binding for a different adenosine A1 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 A1 receptors (Marston et al., 1998). Third, although adenosine A1 and adenosine A2A 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 D2 receptor antagonist eticlopride unlike an adenosine A2A 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
The prominent presynaptic localization of adenosine A1 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 A1 receptors suppress glutamate release include thalamocortical (Fontanet 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 A1 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 A1 receptors.

A significant overlap exists between forebrain distributions of mGlu2 and adenosine A1 receptors in thalamocortical pathways (regulated by 5-HT2A 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 mGlu2 and adenosine A1 receptors in the central nervous system it is parsimonious to propose the working hypothesis that the antidepressant-like effects observed with mGlu2 receptor PAMs (Nikiforuk et al., 2010; Fell et al., 2011) and the adenosine A1 receptor agonist CHA on DRL 72-s behavior is attributable to a suppression of glutamate release at limbic synapses.

Regarding a triangulation of 5-HT2A, mGlu2, and adenosine A1 receptors on operant behavior, the highly selective 5-HT2A 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 the most antidepressant drugs with DRL 72-s behavior (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 mGlu2 and adenosine A1 receptors in the central nervous system it is parsimonious to propose the working hypothesis that the antidepressant-like effects observed with mGlu2 receptor PAMs (Nikiforuk et al., 2010; Fell et al., 2011) and the adenosine A1 receptor agonist CHA on DRL 72-s behavior is attributable to a suppression of glutamate release at limbic synapses.

The prominent presynaptic localization of adenosine A1 receptors throughout the limbic forebrain is consistent with the hypothesis that both mGlu2 receptor PAMs and adenosine A1 receptors in the central nervous system it is parsimonious to propose the working hypothesis that the antidepressant-like effects observed with mGlu2 receptor PAMs (Nikiforuk et al., 2010; Fell et al., 2011) and the adenosine A1 receptor agonist CHA on DRL 72-s behavior is attributable to a suppression of glutamate release at limbic synapses.
tomical distribution of mGlu2 and adenosine A1 receptors, 2) activation of both receptors suppressing glutamate release, 3) activation of both receptors suppressing excitatory synaptic currents induced by 5-HT2A receptor activation, and 4) mGlu2 receptor activation suppressing hallucinogen-induced immediate early gene expression in the medial PFC.

How might CHA, mGlu2 receptor PAMs, and selective 5-HT2A 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, α1-adrenergic receptor antagonists, 5-HT1B/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-HT2A receptor antagonists, CHA, and mGlu2 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-HT2A receptor antagonists, mGlu2 receptor PAMs, and adenosine A1 receptor agonists on DRL behavior highlights an important differential feature compared with the FST, for which adenosine A1 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 A1 receptor agonist and antagonist to examine DRL behavior and 2) the failure to demonstrate a no-effect dose for the adenosine A1 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 A1 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 A1 receptor agonists also decrease rapid eye movement sleep, consistent with the preclinical/clinical action of most antidepressants (Schwerin 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 A1 receptor agonists are not available to test the general hypothesis that decreasing glutamate release in limbic circuitry will result in antidepressant effects in depressed patients because of the peripheral on-target cardiovascular toxicity. However, administration of mGlu2 receptor PAMs could test this hypothesis. Although one mGlu2 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).

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

References
Ardayfio PA, Benvenega MJ, Chaney SF, Love PL, Catlow J, Swanson SP, and Marek GJ (2008) The 5-hydroxytryptamine2A receptor antagonist R(+)-(2,3-dimethoxyphenyl)-1-(4-(4-fluorophenyl)ethyl)-4-piperidinemethanol (M100907) attenuates impulsivity after both drug-induced disruption (dizocilpine) and enhancement (antidepressant drugs) of differential-reinforcement-of-low-rate 72-s behavior in the rat. J Pharmacol Exp Ther 327:891–897.
Fontanez DE and Porter JT (2006) Adenosine A1 receptors decrease thalamic exci-
tation of inhibitory and excitatory neurons in the barrel cortex. *Neuroscience** 137:1177–1184.


