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## Differential actions of adenosine A<sub>1</sub> and A<sub>2A</sub> antagonists on the effort-related effects of dopamine D<sub>2</sub> antagonism

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### Abstract

Adenosine and dopamine receptors in striatal areas interact to regulate a number of different functions, including aspects of motor control and motivation. Recent studies indicate that adenosine A<sub>2A</sub> receptor antagonists can reverse the effects of dopamine (DA) D<sub>2</sub> antagonists on instrumental tasks that provide measures of effort-related choice behavior. The present experiments compared the ability of the adenosine A<sub>2A</sub> antagonist KW6002, the nonselective adenosine antagonist caffeine, and the adenosine A<sub>1</sub> receptor selective antagonist DPCPX, to reverse the behavioral effects of the DA D<sub>2</sub> antagonist haloperidol. For these studies, a concurrent choice procedure was used in which rats could select between lever pressing on a fixed ratio 5 schedule for a preferred food or approaching and consuming a less preferred lab chow that was concurrently available in the chamber. Under baseline or control conditions, rats show a strong preference for lever pressing, and eat little of the chow; IP injections of 0.1 mg/kg haloperidol significantly reduced lever pressing and substantially increased chow intake. The adenosine A<sub>2A</sub> antagonist KW6002 (0.125–0.5 mg/kg IP) and the nonselective adenosine antagonist caffeine (5.0–20.0 mg/kg) significantly reversed the effects of haloperidol. However, the adenosine A<sub>1</sub> antagonist DPCPX (0.1875–0.75 mg/kg IP) failed to reverse the effects of the D<sub>2</sub> antagonist. The rank order of effect sizes in the reversal experiments was KW6002 > caffeine > DPCPX. None of these drugs had any effect on behavior when they were injected in the absence of haloperidol. These results indicate that the ability of an adenosine antagonist to reverse the effort-related effects of a D<sub>2</sub> antagonist depends upon the subtype of adenosine receptor being blocked. Together with other recent results, these experiments indicate that there is a specific interaction between DA D<sub>2</sub> and adenosine A<sub>2A</sub> receptors, which could be related to the co-localization of these receptors on the same population of striatal neurons.

### Keywords

Operant; Reinforcement; Motivation; Behavioral economics; Reward; Decision making; Activation; Depression; Psychomotor slowing; Anergia

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## 1. Introduction

Several lines of evidence indicate that dopamine (DA) and adenosine systems interact in the brain. Striatal areas such as neostriatum and nucleus accumbens are very rich in adenosine A<sub>2A</sub> receptors [13,18,19,33,70], and several papers have reported that there is a functional interaction between striatal DA D<sub>2</sub> and adenosine A<sub>2A</sub> receptors [17-23,27,28,31]. This interaction frequently has been studied in regard to neostriatal motor functions that are related to parkinsonian symptoms [8,20,22,29,32,34,35,46,52,53,59,67,82]. Researchers also have characterized aspects of adenosine A<sub>2A</sub> receptor function related to cognitive processes [76] and motivation [26,44,50]. In particular, several recent studies have focused upon the functional significance of adenosine A<sub>2A</sub> receptors, and the interactions between adenosine and DA receptors, in relation to aspects of behavioral activation and effort-related processes [16,26,44,47,83].

Previous studies have shown that nucleus accumbens DA is a critical component of the brain circuitry involved in behavioral activation and effort-related behavioral processes. Nucleus accumbens DA depletions make rats highly sensitive to ratio requirements in operant schedules [1,7,45,73], and affect response allocation in tasks that measure effort-related choice behavior [58,61-63,66,68]. Some studies in this area have employed maze tasks to assess effort-related choice [9,24,47,65], while others have used a concurrent fixed ratio 5 (FR5)/chow-feeding procedure [38,58,68,73]. In the latter task, rats have a choice between responding on a FR5 lever-pressing schedule for a highly preferred food (i.e., high carbohydrate operant pellets) or approaching and consuming freely available food (i.e., less preferred rodent laboratory chow). Under baseline or control conditions, rats that are trained to respond on this procedure spend most of their time pressing the lever for the preferred food, and eat very little of the concurrently available chow. Low doses of DA antagonists alter choice behavior such that lever pressing for food is suppressed, but chow intake is substantially increased [12,38,58,68,72]. Nucleus accumbens is the DA terminal region most closely associated with these effects [10,11,38,49,68,73]. The actions of DA antagonists or accumbens DA depletions differ substantially from those produced by motivational manipulations such as pre-feeding [38], and appetite suppressant drugs [12,58,72]. These appetite-related manipulations all failed to increase chow intake under conditions that suppressed lever pressing.

Recent papers have reported that intra-accumbens injections of the adenosine A<sub>2A</sub> agonist CGS 21680 produced effects that resembled those of accumbens DA depletions or antagonism, i.e., they impaired performance of operant schedules that had high ratio requirements [44], and they decreased lever pressing and increased chow intake in rats responding on the concurrent choice procedure [26]. In addition, the adenosine A<sub>2A</sub> receptor antagonist MSX-3 has been reported to reverse the effects of DA D<sub>2</sub> antagonists such as haloperidol and eticlopride on tasks that provide measures of effort-related choice behavior, such as the operant concurrent choice task [16,83] and the T-maze choice procedure [47]. The present studies were conducted to investigate the role of DA/adenosine A<sub>2A</sub> receptor interactions in effort-related choice behavior, using the concurrent lever-pressing/chow-feeding procedure. Specifically, these experiments were undertaken to determine if the ability of an adenosine receptor antagonist to reverse the effect of a DA D<sub>2</sub> antagonist is dependent upon the particular subtype of adenosine receptor that was being blocked. In the first group of experiments, three drugs were assessed for their ability to reverse the effects of 0.1 mg/kg of the DA D<sub>2</sub> antagonist haloperidol: the well-known adenosine A<sub>2A</sub> antagonist KW6002 (istradefylline; 0.125–0.5 mg/kg IP), the nonselective adenosine antagonist and minor stimulant caffeine (5.0–20.0 mg/kg), and the adenosine A<sub>1</sub> antagonist DPCPX (0.1875–0.75 mg/kg IP). The fourth experiment studied the effects of the higher doses of KW6002, caffeine, and DPCPX in the absence of haloperidol. In view of the anatomical data demonstrating colocalization of DA D<sub>2</sub> receptors and adenosine A<sub>2A</sub> receptors in striatum and nucleus accumbens, and the well-documented interactions

between these receptors, it was hypothesized that the adenosine A<sub>2A</sub> antagonist would be more effective at reversing the effects of haloperidol than the A<sub>1</sub> selective antagonist.

## 2. Materials and methods

### 2.1. Animals

A total of 33 adult male Sprague—Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in these experiments. They weighed 295–352 g at the beginning of the experiments, and were deprived to 85% of their free-feeding body weight for initial lever press training, but then were allowed modest growth (i.e., an additional 5–10%) throughout the course of the studies. All rats were housed in a climate-controlled animal colony maintained at 23 °C, with 12 h light—dark cycle (lights on 07:00 h), and had access to water ad libitum in their home cages. Animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee, and the studies were conducted according to NIH guidelines.

### 2.2. Behavioral procedures

Behavioral sessions were conducted in operant conditioning chambers (28 cm × 23 cm × 23 cm; Med Associates). Rats were initially trained to lever press on a continuous reinforcement schedule (30-min sessions; 45-mg pellets, Bioserve, Frenchtown, NJ, were used for all operant behavior tests) and then were shifted to the FR5 schedule (30-min sessions, 5 days/week) and trained for several additional weeks. Rats were then trained on the concurrent FR5/chow-feeding procedure. With this task, weighed amounts of lab chow (Lab Diet, 5P00 Prolab RMH 3000, Purina Mills, St. Louis, MO; typically 15–20 g, three large pieces) were concurrently available on the floor of the chamber during the FR5 sessions. At the end of the session, rats were immediately removed from the chamber, and food intake was determined by weighing the remaining food (including spillage). Rats were trained until they attained stable levels of baseline lever pressing and chow intake (i.e., consistent responding over 1200 lever presses per 30 min), after which drug testing began. For most baseline days rats did not receive supplemental feeding, however, over weekends and after drug tests, rats usually received supplemental chow in the home cage. On baseline and drug treatment days, rats normally consumed all the operant pellets that were delivered from lever pressing during each session.

### 2.3. Pharmacological agents and selection of doses

Haloperidol (Sigma Chemical Co., St. Louis, MO) was dissolved in a 0.3% tartaric acid solution (pH 4.0), and this tartaric acid solution also was used as the vehicle control for the haloperidol injections. The adenosine A<sub>2A</sub> antagonist KW6002 was generously provided by Lundbeck Pharmaceuticals (Copenhagen, Denmark), and was dissolved in DMSO and Tween-80 mixed with 0.9% saline (10:10:80% mixture). DPCPX (8-cyclopentyl-1,3-dipropylxanthine) was obtained from Tocris, and was dissolved in a 20% ethanol vehicle. In all experiments, drug treatments were administered IP (see below for descriptions of individual experiments and drug administration schedules).

The dose of haloperidol used to alter choice behavior (0.1 mg/kg IP) was based upon previous research [16,68]. Although higher doses of haloperidol can suppress food intake, this dose did not suppress intake of chow or operant pellets, and did not alter preference between them [68]. Doses of KW6002 and caffeine were determined by unpublished pilot data and on previous research [59]. In addition, a pilot study indicated that 40.0 mg/kg caffeine plus haloperidol actually decreased performance relative to haloperidol alone; for that reasons, a dose range of 5.0–20.0 mg/kg caffeine was selected. Several factors were considered for determining the dose range for DPCPX that was used. In part, it was based upon doses listed in published behavioral studies involving IP administration in rats [2,39,40,47,54]. Furthermore, extensive pilot studies also were performed. In one study, it was determined that

1.5 and 3.0 mg/kg DPCPX did not reverse the effects of haloperidol on lever pressing, and that the 3.0 mg/kg dose actually tended to impair lever pressing in haloperidol-treated rats; this is consistent with a recent T-maze study showing that 3.0 mg/kg DPCPX in combination with haloperidol actually led to worse performance than haloperidol alone [47]. In addition, it was observed that very low doses of DPCPX (i.e., 0.04–0.09 mg/kg IP) also failed to reverse the effects of haloperidol. Based upon all this pilot work, an intermediate dose range of DPCPX (0.1875–0.75 mg/kg) was selected.

## 2.4. Experimental procedures

Rats were trained on the concurrent FR5/chow-feeding procedure (as described above) before drug testing began, and each experiment employed different groups of rats. All five experiments used a within-groups design, with each rat receiving all combined IP drug treatments in their particular experiment in a randomly varied order (one treatment per week, with none of the treatment sequences repeated across different animals in the same experiment). Baseline (i.e., non-drug) sessions were conducted four additional days per week. The specific treatments and testing times for each experiment are listed below.

**2.4.1. Experiment 1: ability of KW6002 to reverse the effort-related effects of haloperidol**—On the test day, rats ( $n = 8$ ) were injected with either tartaric acid vehicle (50 min before testing) plus DMSO/Tween vehicle IP (20 min before testing), 0.1 mg/kg haloperidol IP (50 min before testing) plus DMSO/Tween vehicle IP (20 min before testing), and 0.1 mg/kg haloperidol IP (50 min before testing) plus various doses of KW6002 injected IP (0.125, 0.25 and 0.5 mg/kg; 20 min before testing).

**2.4.2. Experiment 2: ability of caffeine to reverse effort-related effects of haloperidol**—For the drug test days, rats ( $n = 8$ ) were injected with either tartaric acid vehicle (50 min before testing) plus saline vehicle IP (20 min before testing), 0.1 mg/kg haloperidol IP (50 min before testing) plus saline vehicle IP (20 min before testing), and 0.1 mg/kg haloperidol IP (50 min before testing) plus various doses of caffeine injected IP (5.0, 10.0 and 20.0 mg/kg; 20 min before testing).

**2.4.3. Experiment 3: ability of DPCPX to reverse effort-related effects of haloperidol**—Rats ( $n = 10$ ) were injected on drug treatment days with either tartaric acid vehicle (50 min before testing) plus saline vehicle IP (30 min before testing), 0.1 mg/kg haloperidol IP (50 min before testing) plus saline vehicle IP (30 min before testing), and 0.1 mg/kg haloperidol IP (50 min before testing) plus various doses of DPCPX injected IP (0.1875, 0.375 and 0.75 mg/kg; 30 min before testing).

**2.4.4. Experiment 4: effects of KW6002, caffeine and DPCPX in the absence of haloperidol**—Rats ( $n = 5$ ) received IP injections of either saline vehicle, DMSO/Tween vehicle, 20% ethanol vehicle, 0.5 mg/kg KW6002, 20.0 mg/kg caffeine, or 0.75 mg/kg DPCPX 30 min before testing.

## 2.5. Statistical analyses

Total number of lever presses and gram quantity of chow intake from the 30 min sessions were analyzed with repeated measures analysis of variance (ANOVA). When the overall ANOVA was significant, non-orthogonal planned comparisons using the overall error term were used to compare each treatment with the haloperidol plus vehicle control condition. For these comparisons,  $\alpha$  level was kept at 0.05 because the number of comparisons was restricted to the number of treatments minus one (Ref. [37]; pp. 110–139). With this analysis, each condition that combined haloperidol plus adenosine antagonist was compared with its respective haloperidol plus vehicle condition using the planned comparisons. The present analyses used

Systat 7.0, and the factorial ANOVA with trend analysis also tested for interactions across the linear, quadratic and cubic trends. Effect size calculations ( $R^2$  values; [37]) were performed to assess the magnitude of the treatment effect (i.e., the size of the treatment effect sum of squares expressed as the proportion of total sum of squares, which is a marker of the total variance accounted for by treatment variance; for example  $R^2 = 0.3$  reflects 30% of the variance explained) across experiments and measures.

### 3. Results

#### 3.1. Experiments 1–3: KW6002, caffeine and DPCPX combined with haloperidol

KW6002 significantly attenuated the effects of haloperidol on the concurrent lever-pressing/chow-feeding task. The overall treatment effect for lever pressing was statistically significant (Fig. 1A; [ $F(4,28) = 16.6, p < 0.01$ ]). Planned comparisons revealed that haloperidol decreased lever pressing compared to injection of Veh/Veh ( $p < 0.01$ ). KW6002 significantly increased responding in haloperidol-treated rats, with all three doses being significantly different from haloperidol plus vehicle ( $p < 0.05$ ). The overall treatment effect for chow intake also was statistically significant (Fig. 1B; [ $F(4,28) = 11.2, p < 0.01$ ]). Planned comparisons showed that haloperidol increased chow intake compared to injections of Veh/Veh ( $p < 0.01$ ). KW6002 significantly decreased chow intake relative to haloperidol plus vehicle at all three doses ( $p < 0.05$ ).

In experiment 2, caffeine also significantly attenuated the effects of haloperidol on the concurrent lever-pressing/chow-feeding task. The overall treatment effect for lever pressing was statistically significant (Fig. 2A; [ $F(4,28) = 28.8, p < 0.01$ ]). Planned comparisons demonstrated that haloperidol decreased lever pressing compared to injection of Veh/Veh ( $p < 0.01$ ). Caffeine significantly increased responding in haloperidol-treated rats, with all three doses being significantly different from haloperidol plus vehicle ( $p < 0.05$ ). The overall treatment effect for chow intake also was statistically significant (Fig. 2B; [ $F(4,28) = 11.3, p < 0.01$ ]). Haloperidol increased chow intake compared to injections of Veh/Veh ( $p < 0.01$ ). Caffeine significantly decreased chow intake relative to haloperidol plus vehicle at the 10.0 and 20.0 mg/kg doses ( $p < 0.05$ ).

The results of the third experiment showed that DPCPX failed to significantly alter responding in haloperidol-treated rats. The treatment effect for lever pressing was statistically significant (Fig. 3A; [ $F(4,36) = 32.3, p < 0.001$ ]), and as in the other experiments, haloperidol decreased lever pressing compared to injection of Veh/Veh ( $p < 0.01$ ). However, DPCPX combined with haloperidol had no significant effects compared to haloperidol plus vehicle. Similarly, the overall treatment effect for chow intake also was statistically significant (Fig. 3B; [ $F(4,36) = 7.5, p < 0.01$ ]), and planned comparisons revealed that haloperidol increased chow intake compared to injections of Veh/Veh ( $p < 0.01$ ), but there were no significant differences between haloperidol/Veh and haloperidol plus any dose of DPCPX.

#### 3.2. Comparisons across the reversal experiments: effect sizes

In order to make comparisons between the effects of the different adenosine antagonists across multiple experiments, data from the Veh/Veh condition were excluded, and additional ANOVAs were performed. Effect size analyses were conducted based upon these separate ANOVAs performed on each drug reversal experiment (1–3) for both lever pressing and chow intake. There were marked differences in effect sizes between the three reversal experiments (see Table 1). KW6002 had the highest effect size, caffeine was intermediate, and DPCPX had a very small effect size.

### 3.3. Experiment 4: effects of KW6002, caffeine and DPCPX alone

Experiment 4 was a control study that assessed the effects of each vehicle condition used in experiments 1–3, as well as the highest doses of KW6002, caffeine and DPCPX used, in the absence of haloperidol. The means ( $\pm$ S.E.M.) number of lever presses were as follows: saline vehicle: 1250.6 ( $\pm$ 120.2); 20% ethanol/saline vehicle: 1300.0 ( $\pm$ 121.4); DMSO/Tween vehicle: 1227.4 ( $\pm$ 165.7); 0.5 mg/kg KW6002: 1327 ( $\pm$ 179.8); caffeine: 970.6 ( $\pm$ 65.4); 0.75 mg/kg DPCPX: 1567.4 ( $\pm$ 95.6). There were no significant differences between these conditions as determined by repeated measures ANOVA ( $F(5,20) = 2.4$ , n.s.). The means ( $\pm$ S.E.M.) gram quantity of chow intake was as follows: saline vehicle: 2.82 ( $\pm$ 0.65); 20% ethanol/saline vehicle: 2.54 ( $\pm$ 0.80); DMSO/Tween vehicle: 2.02 ( $\pm$ 0.89); 0.5 mg/kg KW6002: 2.10 ( $\pm$ 0.89); caffeine: 2.96 ( $\pm$ 0.27); 0.75 mg/kg DPCPX: 1.80 ( $\pm$ 0.70). As with lever pressing, there were no significant differences between these conditions in terms of chow intake ( $F(5,20) = 2.6$ , n.s.). With both measures, there was a tendency for caffeine alone to decrease responding and increase chow intake, and for DPCPX to increase lever pressing and decrease chow intake. The three vehicle conditions did not significantly differ from each other.

## 4. Discussion

In the experiments presented above, a concurrent choice lever-pressing/chow-feeding task was used to investigate the interaction between adenosine receptor antagonists with different profiles of selectivity and the DA  $D_2$  family antagonist haloperidol. Injection of haloperidol without co-administration of an adenosine antagonist produced a well-documented shift in response allocation; 0.1 mg/kg haloperidol significantly decreased lever pressing and increased chow intake in all experiments. These results are consistent with previously reported findings from experiments that employed systemic administration of numerous DA antagonists with varying degrees of receptor selectivity [12,58,68,72], as well as local DA antagonism in nucleus accumbens [38,49] and accumbens DA depletions [10,11,68,73]. Previous research has shown that the shift from lever pressing to chow intake in rats responding on this task occurs after DA depletions in the nucleus accumbens, but not after depletions in anteroventromedial neostriatum [11]. Moreover, DA depletions in ventrolateral neostriatum produced severe motor impairments that disrupted both lever pressing and feeding [11]. Based upon these results, as well as the outcome of numerous control experiments investigating the effects of various appetite and effort-related manipulations [12,58,66,68,69,72], and studies employing a discrete-trial T-maze choice task [9,47,65], this pattern of findings has been interpreted to indicate that DA antagonists and nucleus accumbens DA depletions are not acting to blunt appetite for food or to suppress primary or unconditioned food reinforcement [3,36,61,68,69]. Rather, these results are widely seen as consistent with the suggestion that DA antagonists and accumbens DA depletions are altering behavioral activation, instrumental response output, response allocation, or effort-related processes [4,25,48,51,<sup>56</sup>,61,68,69,72]. Of course, accumbens DA must participate in effort-related processes in concert with other structures and neurotransmitters [15,16,30,71,80,81], and for that reason the present studies investigated the ability of adenosine antagonists to reverse the effects of the  $D_2$  antagonist haloperidol.

In the first experiment, the adenosine  $A_{2A}$  antagonist KW6002 was able to produce a substantial attenuation of the behavioral effects of the  $D_2$  antagonist haloperidol. Combined administration of KW6002 with haloperidol led to very large increases in lever pressing and decreases in chow intake compared to haloperidol alone. These data are consistent with recent studies indicating that the adenosine  $A_{2A}$  antagonist MSX-3 was able to reverse the effects of the DA antagonists haloperidol [16] and eticlopride [83] in rats responding on the concurrent lever-pressing/chow intake task, and to reverse the suppression of an instrumental barrier climbing response in rats treated with haloperidol [47]. Taken together, these results indicate that adenosine and DA systems interact in the regulation of effort-related functions. Moreover, these observations are

consistent with recent reports that local administration of an adenosine A<sub>2A</sub> agonist into the nucleus accumbens can produce behavioral effects that resemble those of DA antagonists or accumbens DA depletions [26,44].

The nonselective adenosine antagonist caffeine also was able to significantly reduce the effects of haloperidol in experiment 2. Although the effect sizes in the caffeine experiment were generally smaller than those produced by KW6002, it is nevertheless true that this minor stimulant did produce statistically significant effects in haloperidol-treated rats. The present results with caffeine are consistent with previous reports indicating that nonselective adenosine antagonists can reverse the behavioral effects of DA antagonists [79]. Furthermore, the modest effects of caffeine are consistent with reports indicating that caffeine, despite its some-what mixed pattern of binding to adenosine receptors, does show some degree of preference for adenosine A<sub>1</sub> receptors over A<sub>2A</sub> receptors [23]. In marked contrast to the ability of MSX-3, KW6002, and caffeine to reverse the effects of the D<sub>2</sub> antagonist haloperidol, the selective adenosine A<sub>1</sub> antagonist DPCPX did not significantly alter the actions produced by haloperidol. In the dose range tested, DPCPX failed to produce significant changes in lever pressing or chow intake in haloperidol-treated rats. Moreover, pilot experiments indicated that neither higher nor lower doses of DPCPX were effective (see Section 2.3 above). The present results are consistent with several previous studies showing differences between the behavioral effects of adenosine A<sub>1</sub> and A<sub>2A</sub> receptor antagonists [42,43,55]. DPCPX also was reported to be relatively ineffective compared to selective adenosine A<sub>2A</sub> antagonists at producing signs of antiparkinsonian actions in monkeys [79] and rats [6]. Furthermore, the present observations are consistent with a recent report indicating that DPCPX in an IP dose range of 0.75–3.0 mg/kg failed to reverse the effects of 0.15 mg/kg haloperidol in a T-maze task; in fact, the 3.0 mg/kg dose of DPCPX only served to further impair responding in haloperidol-treated rats [47].

The present data, together with other recent results, indicate that there are differential effects of adenosine A<sub>1</sub> and A<sub>2A</sub> receptor antagonists in terms of the extent to which these drugs can reverse the impact of DA D<sub>2</sub> antagonism. Drugs that can exert functional antagonism of A<sub>2A</sub> receptors, including selective A<sub>2A</sub> antagonists such as MSX-3 and KW6002, as well as nonselective adenosine antagonists such as caffeine, can attenuate the effects of DA D<sub>2</sub> antagonists (see Refs. [16,47,83]; experiments 1 and 2 above). In contrast, the adenosine A<sub>1</sub> receptor antagonist DPCPX was not able to do so ([47]; experiment 3 above). In the present studies there were substantial differences in effect sizes between the three reversal experiments (see Table 1); KW6002 had the highest effect size, caffeine was intermediate, and DPCPX had a very small effect size. Furthermore, a recent paper has demonstrated that the adenosine A<sub>2A</sub> antagonist MSX-3 was able to produce a complete reversal of the effects of the highly selective D<sub>2</sub> family antagonist eticlopride in rats responding on the concurrent lever-pressing/feeding task, while it produced only a marginal reversal of the effects of the highly selective D<sub>1</sub> antagonist ecopipam [83]. When considered together, this pattern of results points to a rather important and selective interaction between DA D<sub>2</sub> and adenosine A<sub>2A</sub> receptors. The neurochemical basis of this interaction is not completely clear, however, it is likely that it is related to the pattern of cellular localization of adenosine A<sub>1</sub> and A<sub>2A</sub> receptors in striatal areas, including the nucleus accumbens [17]. Adenosine A<sub>2A</sub> receptors tend to be co-localized on striatal and accumbens medium spiny neurons with DA D<sub>2</sub> receptors (i.e., enkephalin positive medium spiny neurons), and these receptors converge onto the same signal transduction mechanisms and show the capacity for forming heterodimers [17,23,27,31,75]. Therefore, it is reasonable to suggest that adenosine A<sub>2A</sub> receptor antagonists are so effective in reversing the effort-related actions of D<sub>2</sub> antagonists such as haloperidol and eticlopride because of the direct interaction between adenosine A<sub>2A</sub> and DA D<sub>2</sub> receptors located on the same medium spiny neurons. This suggestion is consistent with studies showing that adenosine A<sub>2A</sub> receptor antagonists can reverse the expression of Fos-like immunoreactivity that is induced by D<sub>2</sub> antagonists in medium spiny neurons [52]. In contrast, adenosine A<sub>1</sub> receptors are more likely

to be co-localized with DA D<sub>1</sub> receptors [17], which could help to explain why it is more difficult for adenosine A<sub>1</sub> receptor antagonists to reverse the effects of D<sub>2</sub> receptor blockade. Although DPCPX has generally failed to reverse the behavioral actions of haloperidol, it is possible that DPCPX would be able to reverse T-maze or lever-pressing effects if they were induced by a D<sub>1</sub> antagonist. Future research will investigate this possibility.

In summary, the present data are consistent with previous studies showing that adenosine A<sub>2A</sub> antagonists can reverse the effects of DA D<sub>2</sub> antagonists. This observation has relevance for the potential antiparkinsonian effects of adenosine A<sub>2A</sub> antagonists [8,32,59,67], and also for understanding activational aspects of motivation and effort-related processes [16,47,83]. These findings support the hypothesis that DA and adenosine systems in the brain, possibly in nucleus accumbens, interact in the regulation of instrumental response output and effort-related choice behavior [16,26,44,61]. Furthermore, the present results illustrate the specific nature of the interaction between adenosine A<sub>2A</sub> and DA D<sub>2</sub> receptors, which is likely to be related to the co-localization of these receptors on the same population of striatal and accumbens neurons. Characterization of the neurochemical mechanisms involved in regulating behavioral activation and effort-based choice behavior can shed light on these important facets of motivation, and also may serve to illustrate the relation between activational aspects of motivation (i.e., vigor, persistence and work output) and higher-order processes involved in motor control [61]. Activational aspects of motivated behavior are highly adaptive because they enable organisms to surmount work-related response costs or obstacles that limit access to significant stimuli [57,60,68,78]. In addition, impairments in behavioral activation can be maladaptive. Symptoms such as anergia, psychomotor slowing, and fatigue, which reflect psychopathologies related to behavioral activation in humans, are fundamental aspects of depression and other psychiatric and neurological disorders [5,14,41,61,64,74,77,84]. Research in this area may promote our understanding of the neural mechanisms involved in clinical psychopathologies related to behavioral activation and effort [61,64]. Furthermore, it is possible that A<sub>2A</sub> receptor antagonists could be beneficial for ameliorating the motivational effects of D<sub>2</sub> antagonists that are used clinically, and also for treating other energy-related disorders [64].

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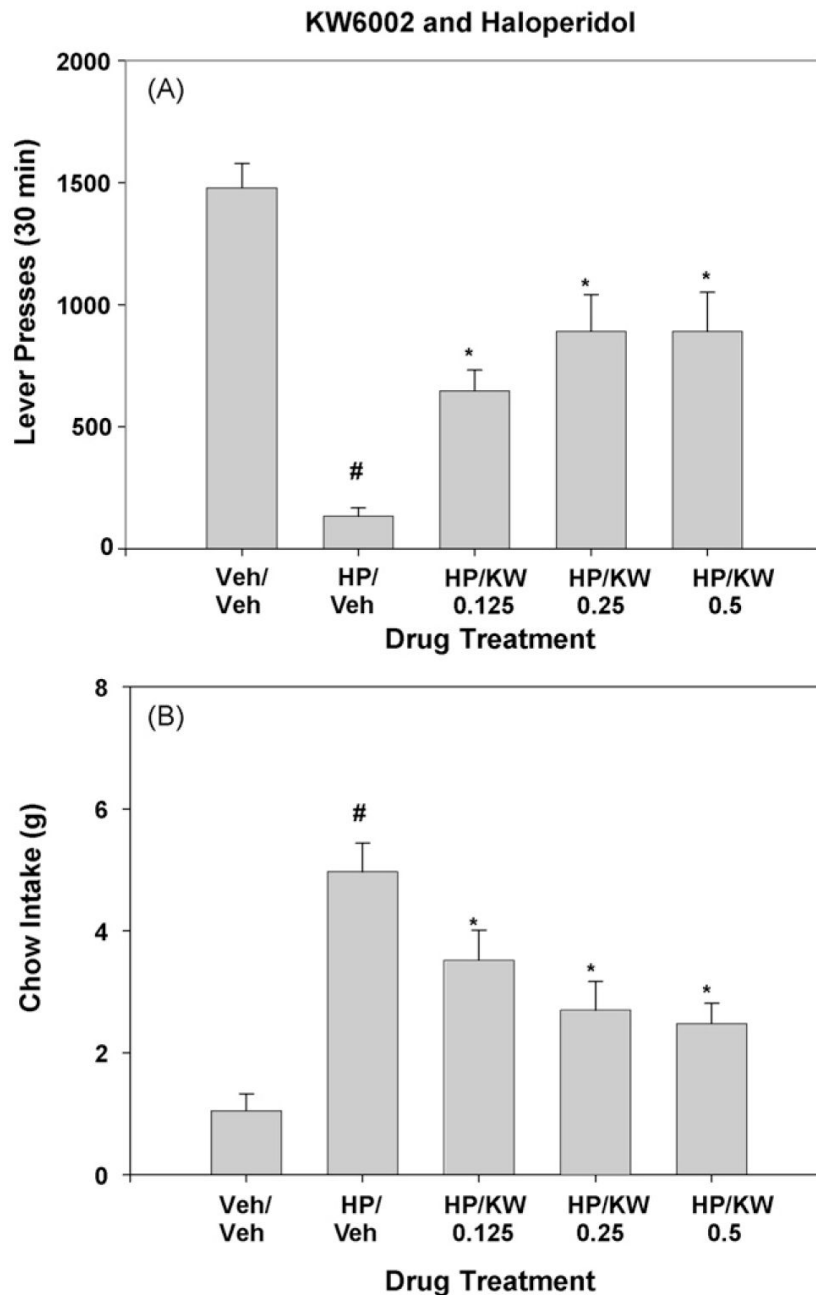
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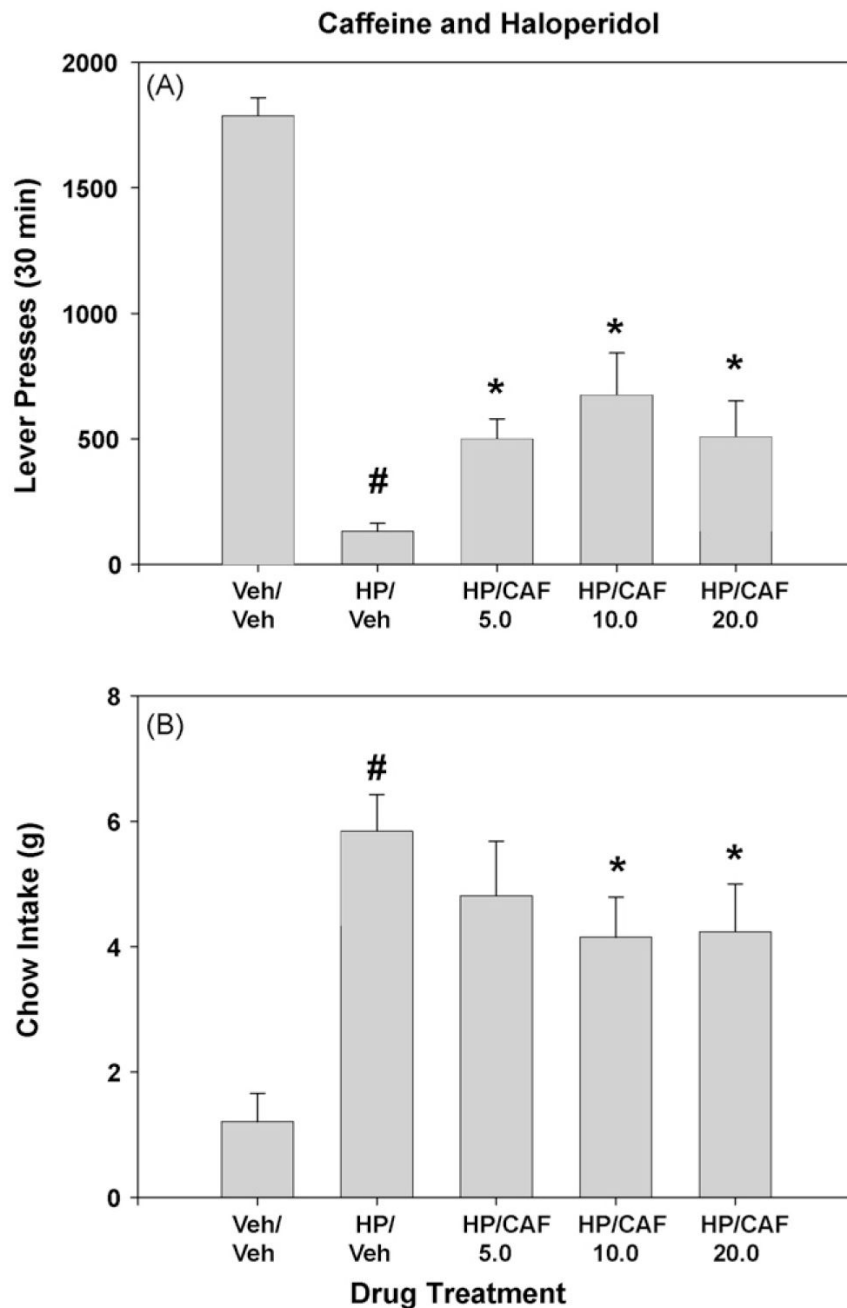
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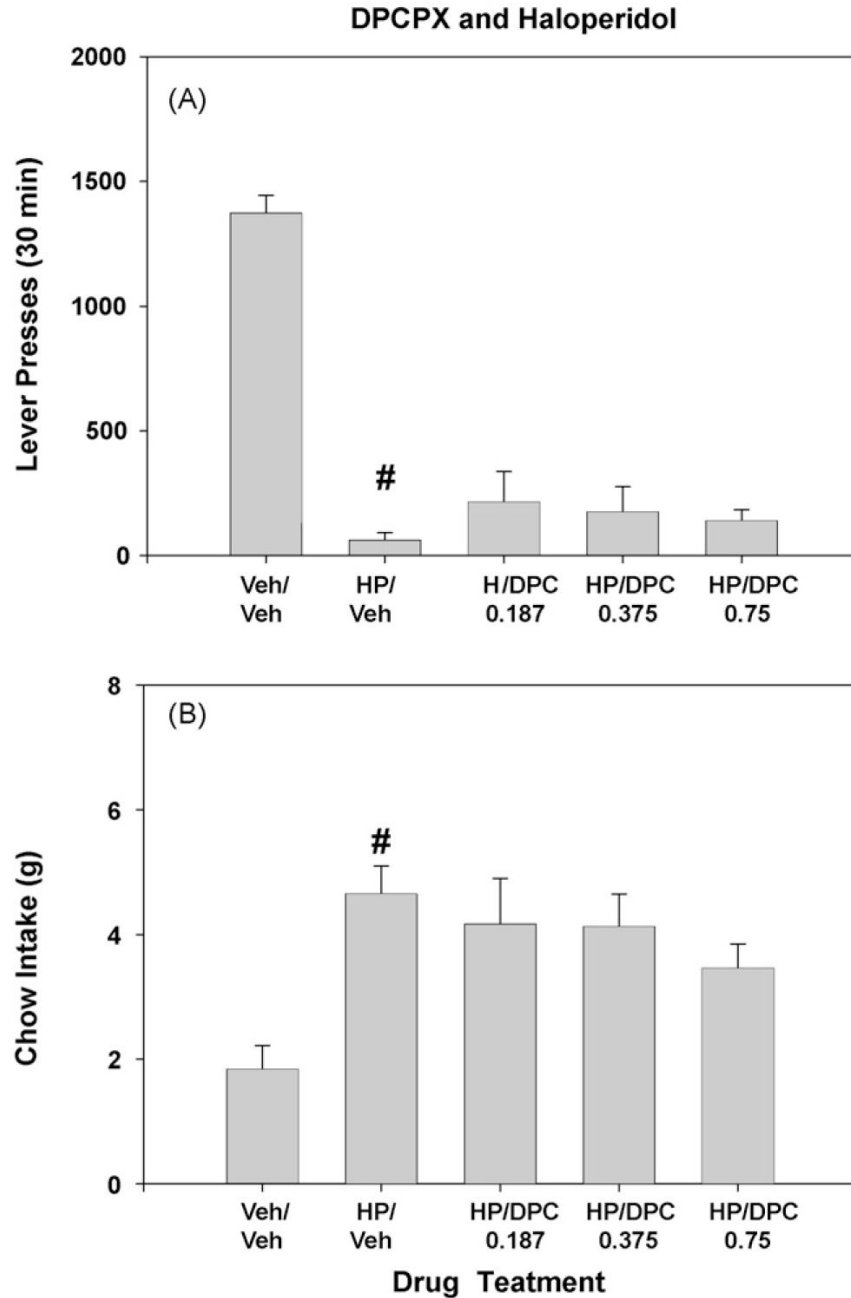
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**Fig. 1.** Effects of the adenosine  $A_{2A}$  antagonist KW6002 in combination with 0.1 mg/kg haloperidol. (A) Mean ( $\pm$ S.E.M.) number of lever presses after treatment with vehicle or haloperidol plus various doses of KW6002 are shown. (B) Mean ( $\pm$ S.E.M.) intake of lab chow (in grams) after treatment with vehicle or haloperidol plus various doses of KW6002 are shown. Veh/Veh (vehicle plus vehicle), HP/Veh (0.1 mg/kg haloperidol plus vehicle), HP/KW 0.125 (0.1 mg/kg haloperidol plus 0.125 mg/kg KW6002), HP/KW 0.25 (0.1 mg/kg haloperidol plus 0.125 mg/kg KW6002), HP/KW 0.5 (0.1 mg/kg haloperidol plus 0.5 mg/kg KW6002). <sup>#</sup> $p < 0.01$ , haloperidol different from vehicle/vehicle, planned comparison; <sup>\*</sup> $p < 0.05$ , different from vehicle plus haloperidol, planned comparison.



**Fig. 2.** Effects of the nonselective adenosine antagonist caffeine in combination with 0.1 mg/kg haloperidol. (A) Mean ( $\pm$ S.E.M.) number of lever presses after treatment with vehicle or haloperidol plus various doses of caffeine are shown. (B) Mean ( $\pm$ S.E.M.) intake of lab chow (in grams) after treatment with vehicle or haloperidol plus various doses of caffeine are shown. Veh/Veh (vehicle plus vehicle), HP/Veh (0.1 mg/kg haloperidol plus vehicle), HP/CAF 5.0 (0.1 mg/kg haloperidol plus 5.0 mg/kg caffeine), HP/CAF 10.0 (0.1 mg/kg haloperidol plus 10.0 mg/kg caffeine), HP/CAF 20.0 (0.1 mg/kg haloperidol plus 20.0 mg/kg caffeine). <sup>#</sup> $p < 0.01$ , haloperidol different from vehicle/vehicle, planned comparison; <sup>\*</sup> $p < 0.05$ , different from vehicle plus haloperidol, planned comparison.



**Fig. 3.** Effects of the adenosine A<sub>1</sub> antagonist DPCPX in combination with 0.1 mg/kg haloperidol. (A) Mean ( $\pm$ S.E.M.) number of lever presses after treatment with vehicle or haloperidol plus various doses of DPCPX are shown. (B) Mean ( $\pm$ S.E.M.) intake of lab chow (in grams) after treatment with vehicle or haloperidol plus various doses of DPCPX are shown. Veh/Veh (vehicle plus vehicle), HP/Veh (0.1 mg/kg haloperidol plus vehicle), HP/DPC 0.187 (0.1 mg/kg haloperidol plus 0.1875 mg/kg DPCPX), HP/DPC 0.37 (0.1 mg/kg haloperidol plus 0.375 mg/kg DPCPX), HP/PDC 0.75 (0.1 mg/kg haloperidol plus 0.75 mg/kg DPCPX). <sup>#</sup> $p < 0.01$ , haloperidol different from vehicle/vehicle, planned comparison.



**Table 1**Effect size calculations ( $R^2$  values) for the reversal experiments.

<b>Drug</b>	<b>Lever pressing</b>	<b>Chow intake</b>
KW6002	0.49	0.40
Caffeine	0.21	0.11
DPCPX	0.06	0.08