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Adenosine antagonists reverse the cataleptic effects of haloperidol: Implications for the treatment of Parkinson's disease

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ABSTRACT

The effects of adenosine antagonists were compared in two rodent models of Parkinsonian symptoms. In the first experiment the dopamine D_2 antagonist, haloperidol, was used to induce catalepsy. It was found that treatment with the non-selective adenosine antagonist caffeine significantly reduced catalepsy at each dose. Treatment with the selective A_1 antagonist CPT also produced a significant reduction in catalepsy, as did treatment with the selective A_{2A} antagonist SCH58261. In the second experiment haloperidol was used to suppress locomotor activity in an open field test. Treatment with caffeine significantly increased locomotion reduced by haloperidol, but not at all doses tested. Treatment with CPT also increased haloperidol-suppressed locomotor activity in dose-dependent manner. Surprisingly, treatment with SCH58261 did not significantly increase locomotor activity in animals treated with haloperidol at any dose tested. While some of these results were unexpected, the overall pattern suggests that adenosine antagonists would be useful as therapies for Parkinsonian patients as they appear to increase movement. The results also suggest that in acute timelines A_1 antagonists may be more beneficial than previously supposed.

Parkinson's disease (PD) is the most common movement disorder in the United States today, affecting approximately 1 million individuals (Oertel, 1995; Olanow, 2004). The cause of idiopathic PD appears to be the loss of dopamine-containing (dopaminergic) neurons in the substantia nigra pars compacta (SNc; Montastruc et al., 1994), although Parkinsonism can also result from the long-term use of certain neuroleptic drugs. The resulting disruption of neurochemical function in the basal ganglia produces the symptoms of akinesia, bradykinesia and tremor that characterize Parkinson's disease.

While dopamine (DA) has long been the neurotransmitter most closely associated with PD, several other neurotransmitters active in the basal ganglia are also affected. Recent studies have indicated that adenosine neurons modulate the activity of striatal projection neurons, and are thus in a key position to affect the overall function of the basal ganglia (Ferre et al., 1993; Golembiowska and Dziubina, 2004; Morelli and Pinna, 2001). There are several adenosine subreceptors, but binding data reveal that the A_1 and A_{2A} subtypes are most prevalent in the basal ganglia. While central A_{2A} receptors are expressed almost exclusively in the striatum (Ferre et al., 1993; Pinna et al., 2005; Svenningsson et al., 1997; Tanganelli et al., 2004), A_1 receptors have a relatively high expression throughout the brain; highest densities are found in the stratum oriens, hippocampus, cerebral cortex, striatum and thalamus (Fastbom et al., 1986, 1987a,b; Svenningsson et al., 1997). Due to the anatomical specificity of A_{2A} receptors to the striatum (Svenningsson et al., 1998), as well as the colocalization of A_{2A} receptors and D_2 receptors on medium sized spiny neurons that give rise to the striatopallidal output pathway (Fredholm and Svenningsson, 2003; Fredholm et al., 2003; Golembiowska and Dziubina, 2004), A_{2A} receptors have become a very attractive therapeutic target for managing the symptoms of PD.

It is hypothesized that the striatopallidal pathway and the striatonigral pathway work together in a complex, coordinated fashion to create smooth, controlled movement. Activation of the striatonigral pathway has been hypothesized to promote movement, while activation of the striatopallidal pathway may result in the suppression of movement (Wichmann and DeLong, 2003). A_{2A} receptors and D_2 receptors act in an antagonistic manner; it is believed that a major role of dopamine is to antagonize tonically active signaling via A_{2A} receptors (Tanganelli et al., 2004; Vortherms and Watts, 2004). Dopamine loss would lead to unopposed adenosine signaling (Fredholm and Svenningsson, 2003), resulting in over-activity of the striatopallidal pathway, and excess inhibition of movement.

Evidence from epidemiological studies has found a strong inverse relationship between coffee drinking and prevalence of Parkinson's disease within many populations (Gale and Martyn, 2003). Additionally, it was found that patients with PD who drank coffee regularly had

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less pronounced symptoms of the disease compared to those with PD who did not. Furthermore, caffeine and other adenosine receptor antagonists have been shown to decrease the symptoms of PD (Fredholm et al., 2003). Recent studies have demonstrated the effectiveness of selective adenosine antagonists as an adjunctive treatment to $_L$-DOPA therapy (Fuxe et al., 2008; Gottwald and Aminoff, 2008; Hauser et al., 2008; Jenner, 2005; Kase et al., 2003) , and studies with rodents have shown that A2A antagonists effectively reduce catalepsy and reverse locomotor activity suppressed by D2 antagonists (Antoniou et al., 2005; Correa et al., 2004; Malec, 1997; Moo-Puc et al., 2003; Salamone et al., 2008b). However, it remains unclear whether the beneficial effects of adenosine manipulation extend equally to antagonism at one or both types of adenosine receptor.

The purpose of the current study was to investigate the effects of adenosine antagonists in two rodent models of PD. Locomotion is a behavioral test that has historically been used to assess the effects of DA antagonists, and has been shown to be effectively suppressed by both D_1 and D_2 antagonists (Beninger, 1983; Clow et al., 1979; Fishman et al., 1983; Janssen et al., 1966; Molloy et al., 1986). Catalepsy has traditionally been used to model akinesia, and is usually induced by the antagonism of D_2 receptors (De la Cruz Lopez and Santamaria, 1996; De Ryck et al., 1980; Fischer et al., 2002; Svenningsson et al., 1998; Wolgin, 1985). Doses of haloperidol ranging from 1.0 to 10.0 mg/kg have been used in rats with various behavioral effects noted including exaggerated bracing, disrupted hopping and, in the case of 5.0 mg/kg or more, almost complete akinesia (Wolgin, 1985), as well as profound immobility (De la Cruz Lopez and Santamaria, 1996); 3.0 mg/kg induced catalepsy as measured by bar-time, but did not induce muscular rigidity response as measured by hindlimb flexion (Fischer et al., 2002).

In the current study, systemic administration of the D_2 antagonist haloperidol was used to induce Parkinsonian symptoms; a dose of 0.75 mg/kg was used to suppress locomotor activity and a dose 5.0 mg/kg was used to induce catalepsy. The effects of three adenosine antagonists (the non-selective adenosine antagonist caffeine, the selective A_1 antagonist CPT and the selective A_{2A} antagonist SCH58261) were evaluated. Because the catalepsy model reflects a higher degree of DA dysfunction, it was predicted that adenosine antagonism would be more effective at relieving catalepsy than restoring locomotor activity, as the modulation of adenosinergic activity was expected to have a greater impact. It was also predicted that in both models antagonism of adenosine A_{2A} receptors would restore behavior more effectively than antagonism of A_1 receptors. A_{2A} receptors are almost exclusively located on neurons in the striatopallidal pathway, and Parkinsonian symptoms are associated primarily with dysfunction of this pathway; therefore blockade of A_{2A} receptors should be more critical to restoration of normal behavior. In addition, as noted above both catalepsy and locomotor suppression will be induced using the D2 antagonist haloperidol. As A2A receptors are colocalized with D2 receptors on the striatopallidal neurons it is expected that manipulation of A2A receptors will be more instrumental to restoring behavior (Ferre et al., 1993).

1. Methods

1.1. Animals

Sixty male albino Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing between 250 and 300 g were used in these studies. Animals were group housed in a temperature- and light-controlled room (12 hour light–dark cycle with lights on at 0700.), had *ad lib* access to food and water and were cared for in accordance with University policy and IACUC guidelines. Each individual experiment used a separate group of 10 animals. Each experimental protocol was approved by the California State University, Fullerton IACUC committee.

1.2. Drugs

Haloperidol (Sigma; St. Louis, MO; dissolved in 0.03% tartaric acid) was used to induce Parkinsonian symptoms. To reverse the effects of haloperidol the following drugs were used: the non-selective adenosine antagonist caffeine (Sigma; dissolved in 0.03% tartaric acid), the selective A_1 antagonist 8-cyclopentyl-1,3-dimethlyxanthine (CPT; Sigma; dissolved in 0.9% NaCl) and the selective A_{2A} antagonist 2-(2-Furanyl)-7-(2-phenylethyl-7H-pyrazolo[4,3-e][1,2,4] triazolo[1,5-c]pyrimidin-5-amine (SCH58261; Tocris, Ellisville, MO; dissolved in a 1:3:7 mixture of DMSO, Tween 80 and 0.9% NaCl). All drugs were administered intraperitoneally. In the catalepsy experiments the following doses were used: haloperidol, 0.0 and 5.0 mg/kg; caffeine, 0.0, 10.0, 20.0 and 40.0 mg/kg; CPT, 0.0, 2.0, 4.0 and 8.0 mg/kg; SCH58261 0.0, 2.5, 5.0 and 10.0 mg/kg. In the open field experiments the following doses were used: haloperidol, 0.0 and 0.75 mg/kg; caffeine, 0.0, 10.0, 20.0 and 40.0 mg/kg; CPT, 0.0, 2.0, 4.0 and 8.0 mg/kg; SCH58261 0.0, 2.5, 5.0 and 10.0 mg/kg. The doses of caffeine, SCH58261 and CPT, as well as the wait times after the injections, were based on previous behavioral studies (Pinna et al., 2007; Simola et al., 2004).

1.3. Apparatus

1.3.1. Catalepsy

Catalepsy was determined through the use of a standard bar test. The apparatus consisted of a metal bar (.04 cm in diameter × 25.0 cm long) standing 10.0 cm. high on a wooden platform.

1.3.2. Open field

A 112.5 cm × 112.5 cm × 45 cm box (open top) fashioned from 1.25 cm plywood was used to assess the total movement made by the animals. The inside surfaces of the box were painted black and a 5X5 square grid was marked on the bottom of the box with white 1.25 cm waterproof tape. Each square section measured 22.5 cm by 22.5 cm.

1.4. Procedure

1.4.1. Catalepsy

Each adenosine antagonist was tested using a separate group of drug naïve animals ($n = 10$; total $N = 30$). All data were collected by trained observers blind to the condition of the animal and took place once a week to allow for complete drug wash out between testing sessions. Observations were made in a lit room during day time hours. One hundred and twenty minutes prior to testing the animals received injections of haloperidol (or vehicle), followed 60 min later by injection of one of the adenosine antagonists (or vehicle). The animals were placed on the apparatus such that their forepaws rested on the bar and their hind quarters on the platform. After the animal was positioned properly the experimenter released their hold and began timing. Catalepsy was measured by the time the animal maintained its position on the bar. Time was stopped when the animal fully removed both paws from the bar. Animals were observed for a maximal time of 2 min (120 s). Following assessment the animals were returned to their home cages.

1.4.2. Open field

Each adenosine antagonist was tested using a separate group of drug naïve animals ($n = 10$; total $N = 30$). All data were collected by trained observers blind to the condition of the animal and took place once a week to allow for complete drug wash out between testing sessions. Pilot testing had indicated that allowing the animals to habituate prior to each testing session produced very low baseline locomotor activity. Thus, it was decided that one week prior to the first test day each animal would spend 5 min in the open field to habituate to the new environment. Throughout the experiment before each

animal was placed into the open field, the box was wiped down entirely with isopropyl alcohol to eliminate odor traces. Testing was conducted in a dark room under red light illumination. Fifty minutes prior to testing the animals received injections of haloperidol (or vehicle), followed 30 min later by injection of one of the adenosine antagonists (or vehicle). The animals were placed in a corner of the open field (the same corner was used for all trials) and observed for 5 min. Immediately upon placing the animal in the open field the observer began to count the animal's movement with a mechanical hand counter. A movement was counted when the animal crossed a grid line with all four paws. If the animal crossed a square diagonally (going over the intersection of two grid lines) it was counted as only one movement. After the 5-minute observation period, the animal was removed from the open field and returned to its home cage.

1.5. Design

All experiments employ an incomplete 2x4 repeated measures design, wherein the two independent variables are 1) dose of haloperidol, and 2) dose of adenosinergic compound. Haloperidol had two levels: 0.0 mg/kg (vehicle) and an experimental dose (as listed above for each experiment). Each adenosine antagonist had four levels: 0.0 mg/kg (vehicle) and three experimental doses (as listed above for each experiment). The design is incomplete as it was decided that testing the 0.0 mg/kg haloperidol dose in combination with the experimental doses of the adenosine antagonists would not yield data that would provide information regarding the abilities of these compounds to reverse haloperidol-induced motor deficits. For the purpose of statistical analysis, the levels of the two independent variables were combined to yield five distinct drug "treatments" which were compared using a repeated measures ANOVA test. For example, the study investigating the effects of haloperidol and caffeine on catalepsy had 5 treatment levels: 1) 0.0 mg/kg haloperidol + 0.0 mg/kg caffeine (control), 2) 5.0 mg/kg haloperidol + 0.0 mg/kg caffeine (haloperidol alone), 3) 5.0 mg/kg haloperidol + 10.0 mg/kg caffeine, 4) 5.0 mg/kg haloperidol + 20.0 mg/kg caffeine and 5) 5.0 mg/kg haloperidol + 40.0 mg/kg caffeine (see Table 1). Animals were randomly assigned each week to a specific condition, and each animal received each treatment condition throughout the course of the study. Data for each adenosine antagonist was analyzed using separate repeated measures one-way ANOVA and post-hoc comparisons were made using t-tests to examine differences between individual treatment groups within a given experiment. Family-wise error was corrected for using the Bonferroni adjustment.

2. Results

2.1. Catalepsy

Pretreatment with haloperidol and caffeine produced a significant change in the duration of catalepsy ($F(4, 36) = 31.557$, $p<0.001$; see Fig. 1). Post-hoc comparisons showed that the haloperidol-alone group ($M = 117.00$; treatment 2) stayed on the bar significantly longer than the control group ($M = 2.90$; treatment 1), demonstrating the effective induction of catalepsy ($p<0.05$). Comparisons of the individual doses of caffeine with the haloperidol-alone group revealed a significant reduction of catalepsy at each dose. In addition, the 20.0 mg/kg caffeine treatment ($M = 49.60$) and 40.0 mg/kg caffeine treatment ($M = 20.60$) groups were not significantly different from the control group, indicating that treatment with the two highest doses of caffeine reduced catalepsy to baseline levels.

Table 1
Experiment 1 treatment design.

	Caffeine			
Haloperidol	0.0 mg/kg	10.0 mg/kg	20.0 mg/kg	40.0 mg/kg
0.0 mg/kg (vehicle)	Treatment 1			
5.0 mg/kg	Treatment 2	Treatment 3	Treatment 4	Treatment 5

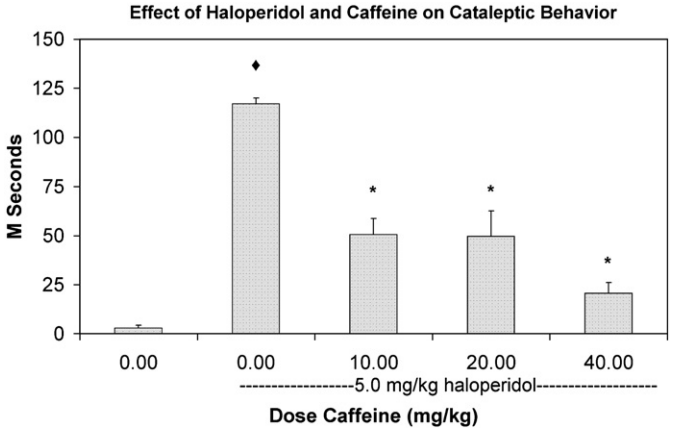

Fig. 1. Effects of caffeine on haloperidol-induced catalepsy. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$. *Hal/Caff treatment group significantly different from Hal/Veh, $p<.05$.

Treatment with CPT also showed a significant overall reduction in catalepsy ($F(4, 36) = 33.77$, $p<0.001$; see Fig. 2). Post-hoc comparisons ($p = .05$) of the experimental doses of CPT revealed that the 4.0 mg/kg dose ($M = 41.90$) and 8.0 mg/kg dose ($M = 37.50$) were significantly different from the haloperidol-alone group ($M = 111.10$), but that the 2.0 mg/kg dose ($M = 78.50$) was not. Mean catalepsy times for all doses of CPT were not significantly different from the control condition ($M = 0.30$), indicating that none of the doses returned the behavior to baseline levels.

Treatment with SCH58621 produced similar significant decreases in catalepsy ($F(4, 36) = 12.097$, $p<0.001$; see Fig. 3). Post-hoc comparisons ($p = .05$) demonstrated that treatment with either 2.5 mg/kg ($M = 28.60$) or 10.0 mg/kg ($M = 41.80$) SCH58621 produced significant decreases in catalepsy compared to the haloperidol-alone condition. In addition, at these doses measures of catalepsy did not differ significantly from the control group, indicating that the behavior was restored to baseline levels.

2.2. Open field

Analysis of the data revealed that pretreatment with haloperidol and caffeine produced a significant difference in the total amount of

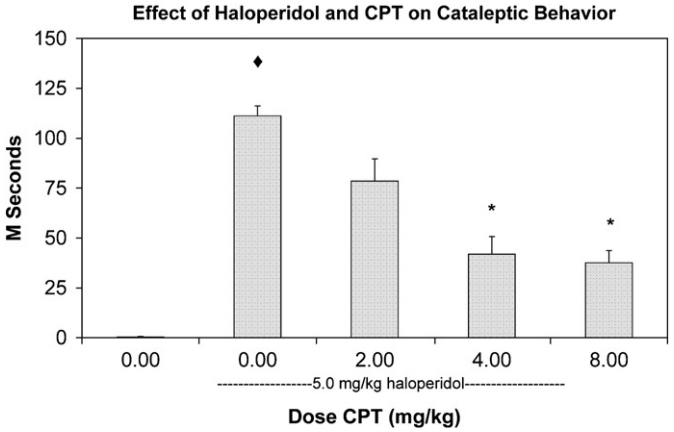

Fig. 2. Effects of CPT on haloperidol-induced catalepsy. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$. *Hal/CPT treatment groups significantly different from Hal/Veh, $p<.05$.

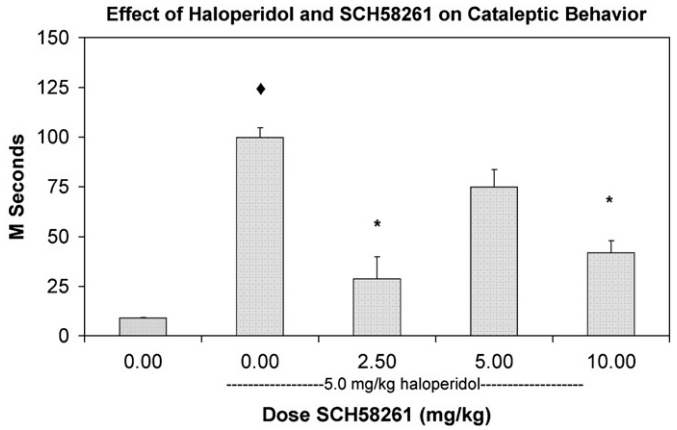

Fig. 3. Effects of SCH582561 on haloperidol-induced catalepsy. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$. *Hal/SCH58261 treatment groups significantly different from Hal/Veh, $p<.05$.

locomotion made by the animals in the open field $F(4,36)=17.30$, $p<.001$ (see Fig. 4). As predicted, post hoc comparisons ($p=.05$) showed that the 0.75 mg/kg dose of haloperidol produced a significant decrease in locomotor activity ($M=11.30$) as compared to the 0.0 mg/kg dose ($M=89.60$). Caffeine significantly restored movement levels at the 20.0 mg/kg dose ($M=36.50$), but failed to do so at the 10.0 mg/kg ($M=32.30$) and 40.0 mg/kg dose ($M=22.60$). Somewhat surprisingly, the adenosine antagonist CPT successfully restored locomotor activity in the animals in a dose-dependent manner ($F(4,36)=8.406$, $p<.001$; see Fig. 5). Interestingly, there was no significant difference between the animals in the control condition (treatment condition 1; $M=109.5$) and the animals that received 8.0 mg/kg CPT (treatment condition 5; $M=82.75$), indicating that at the highest dose of CPT activity was fully restored to baseline levels. Also surprisingly, the selective adenosine A_{2A} antagonist SCH58261 did not succeed in restoring locomotor activity. Although the overall ANOVA was significant ($F(4,36)=9.105$, $p<.001$; see Fig. 6), this reflects the significant difference between the control condition (treatment condition 1; $M=59.30$) and the haloperidol-alone condition (treatment condition 2; $M=6.2$). Contrary to prediction, none of the experimental doses of SCH58261 were successful at restoring movement suppressed by haloperidol.

3. Discussion

The present study demonstrated that the adenosine antagonists caffeine, CPT, and SCH58621 were each able to dose dependently reverse catalepsy. Although data showed significant reversal by each of the adenosine antagonists, caffeine and the selective A_{2A} antagonist SCH58621 were the most effective at reversing the cataleptic state and restoring behavior to baseline measures. Caffeine at each of the experimental doses was able to decrease the time spent on the bar to times similar to baseline measures. Similarly, in the SCH58621 group catalepsy measures for animals treated with each of the experimental doses did not differ significantly from the measures of the control group. CPT was able to reverse the cataleptic state although it was not able to restore behavior to control levels. These findings are consistent with many other studies finding A_{2A} antagonism to be an effective method by which to reduce catalepsy (Antoniou et al., 2005; Kafka and Corbett, 1996; Malec, 1997; Moo-Puc et al., 2003). It is believed that this increase in effectiveness is due to the selective co-localization of the A_{2A} receptor with the D_2 receptor on striatopallidal neurons. In addition, it has been found that within the brains of Parkinsonian patients the number of adenosine A_{2A} receptors increases in the substantia nigra pars reticulata as well (Hurley et al., 2000). Given the oppositional effects of A_{2A} receptor action on dopamine synthesis and release (Bibbiani et al., 2003), it may be that blockade of A_{2A} receptors in the SNr would facilitate the production and release of DA from the remaining DAergic neurons.

Although the A_1 receptor is partially co-localized with D_1 receptors, it may be that the pairing is not selective enough to have the potent effects of the A_{2A}/D_2 coupling. It has been proposed that A_1 receptors have a reduced effect on akinesia due to the extensive locality of A_1/D_1 interaction on striatoentopeduncular neurons (Hauber, 1998a); an important fact given the evidence indicating the striatopallidal projection as being the most critical for readiness and initiation of movement. Although A_1 receptor density is seen most heavily in the basal ganglia network, the locality of the receptors is more sporadic and has fewer co-localities with dopamine D_1 receptors. This placement allows adenosine on A_1 receptors to exert some influence; however, the A_{2A} receptor should be far more instrumental in the reduction of a cataleptic state. Also, A_{2A} and A_1 receptors have some interaction despite their differing locations due to dendritic crossover between nuclei, especially via the minimal co-localization of A_{2A}/D_1 receptors (Chen et al., 2000). Hence, A_{2A} antagonists may be inadvertently facilitating dopamine efficacy through the striatonigral pathway as well as the striatopallidal pathway.

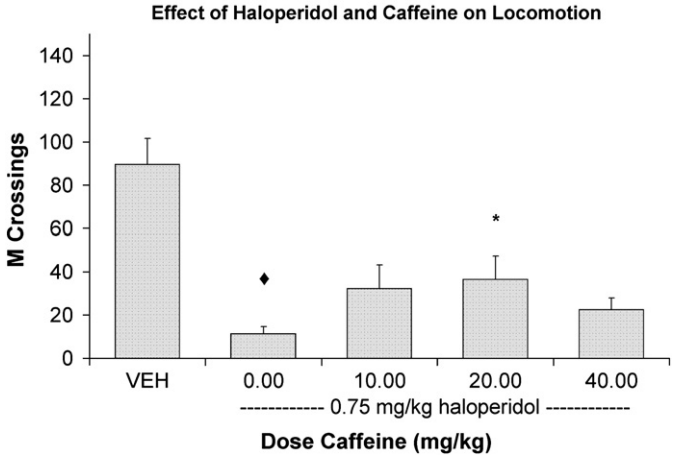

Fig. 4. Effects of caffeine and haloperidol on locomotor activity. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$. *Hal/Caff treatment groups significantly different from Hal/Veh, $p<.05$.

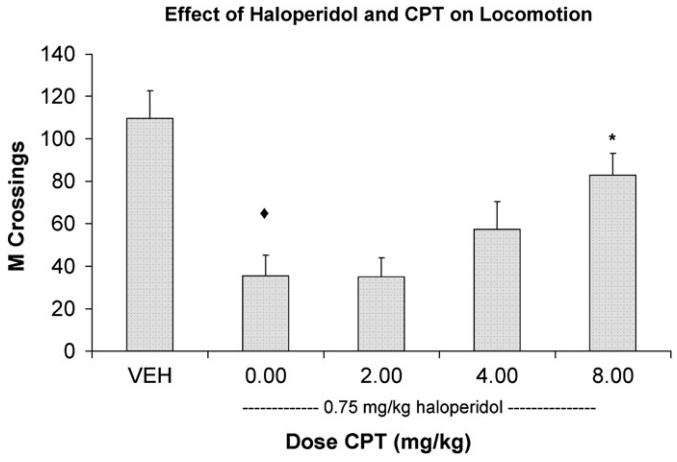

Fig. 5. Effects of CPT and haloperidol on locomotor activity. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$. *Hal/CPT treatment groups significantly different from Hal/Veh, $p<.05$.

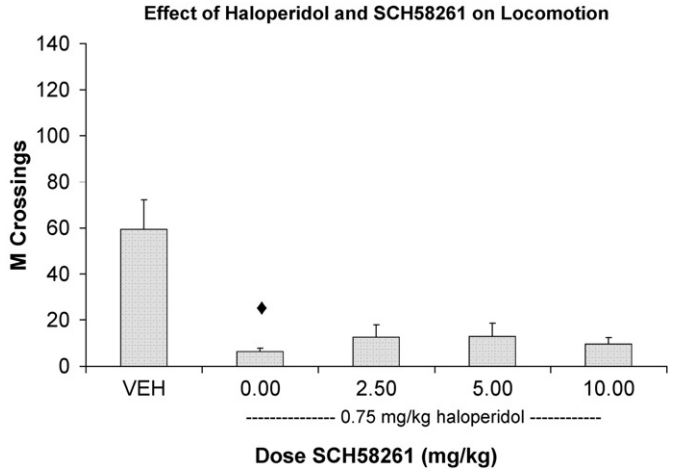

Fig. 6. Effects of SCH58261 and haloperidol on locomotor activity. Results shown as means ± standard errors of measurement (S.E.M.). ♦Hal/Veh significantly different from Veh/Veh, $p<.01$.

Treatment with adenosine antagonists may also be beneficial as it has been suggested that they may prevent the development of dyskinesias in patients receiving L-DOPA therapy (Schwarzschild et al., 2006). It has been found that hemi-parkinsonian rats treated daily with a low dose of L-Dopa and the A_{2A} antagonist SCH58261 did not develop the sensitized rotational response that was seen in rats treated with L-Dopa alone (Pinna et al., 2001). Additionally, in studies investigating A_{2A} receptor knockout mice, it was found that chronic administration of L-DOPA to the animals lacking the A_{2A} receptor site did not produce dyskinesia, suggesting that the activation of this receptor site is a requisite to the development of L-DOPA induced motor complications (Alfinito et al., 2003). Dyskinesias are part of what are referred to as "type A" effects, that is, those adverse effects that are considered to be part of the pharmacokinetic properties of the drug (e.g. L-DOPA) and are unavoidable (Rascol et al., 2003). Initial data from clinical trials has shown that A_{2A} antagonism may be able to alleviate dyskinesias in patients who have developed L-DOPA induced motor complications (Schwarzschild et al., 2006). Further studies have indicated that A_{2A} antagonism may have peripheral anti-depressant and anti-inflammatory effects (El Yacoubi et al., 2001; Sitkovsky et al., 2004).

The current data support the idea that adenosine antagonism is effective in relieving the Parkinsonian symptoms of catalepsy and muscular rigidity. SCH58621, the A_{2A} specific antagonist, demonstrated a higher degree of potency, maintaining its effectiveness at lower doses. As with any pharmacological treatment, lower doses are preferred in order to keep unwanted side effects at bay. Although CPT, the A_1 specific antagonist, was able to show some reversal of catalepsy, the peripheral benefits of antagonism at the A_{2A} site make this the most desirable adjunctive therapeutic approach.

The locomotion experiments produced unexpected and interesting results. As predicted, administration of caffeine reversed locomotor suppression; however this was only seen at the 20.0 mg/kg dose. In addition, the A_{2A} antagonist did not restore locomotion while the A_1 antagonist did. While it was initially predicted that treatment with the A_{2A} receptor antagonists would create the greatest restoration of movement in the animals, the results of the present experiment indicate that the A_{2A} antagonist was actually the least effective in restoring movement. This finding is perplexing; review of the literature indicates that other investigators have found that A_{2A} antagonists effectively restore locomotor activity (Correa et al., 2004; Salamone et al., 2008b). Additionally, the current model of the dopamine-adenosine interaction suggests that the A_{2A} receptor generally affects only the dopamine D_2 subreceptor and works primarily through the striatopallidal pathway. As the striatopallidal pathway is thought to be the most affected by antagonism of striatal dopamine receptors it is reasonable to suppose that blockade of A_{2A} receptors would be more effective than blockade of A1 receptors for restoring normal movement (Ferre et al., 1993). However, compared to caffeine and CPT, SCH 58261 had virtually no effect on movement in the open field design. Several possible reasons exist for the observed results. Previous studies have successfully reversed haloperidol-induced suppression of locomotion with A_{2A} antagonists (Correa et al., 2004; Ishiwari et al., 2007; Salamone et al., 2008a) however these studies have also used a lower dose of haloperidol (0.5 mg/kg). Additionally, although the animals were randomly assigned to the three drug treatment groups the baseline (control) levels of locomotion in the SCH58261-treated animals were markedly lower than those of the either the CPT- or haloperidol-treated animals. It is possible that these animals were less inclined to move due to random and non-controlled parameters. Given the difference in baseline locomotor activity in the SCH58261 group and the fact that the results of this experiment are contradictory to previous results it is important to interpret them cautiously.

Treatment with the A_1 antagonist produced a dose-dependent increase in locomotion, with the highest dose (8.0 mg/kg) restoring activity to baseline levels. While A_1 antagonists have been shown to alleviate symptoms of Parkinsonism in some experiments, in other experiments these drugs have been shown to be ineffective (Hauber, 1998b; Varty et al., 2008). However, it has been proposed that while both A_1 and A_{2A} antagonism contribute to caffeine's motor stimulatory effects, A_1 antagonism may play a greater role when administered acutely while A_{2A} antagonism makes a greater contribution following chronic caffeine administration due to the development of tolerance at A_1 receptors (Antoniou et al., 2005; Karcz-Kubicha et al., 2003).

Additionally, the open-field measure is not unique to this specific area of study. It has also been used to test levels of subjective anxiety in animals (Calabrese, 2008; Kliethermes, 2005). Therefore, it is possible, that the reason for these unexpected results is due to a possible confound between anxiety level and motor activity. Several studies have indicated that adenosine antagonists are anxiogenic, and thus might induce more activity (Guttmacher et al., 1983; Nehlig et al., 1992; Thorsell et al., 2007). When using the open field to measure anxiety it is common to compare the total time spent in the periphery of the open field to the total time spent in the center; the present study did not distinguish between these two measures, thus it is not possible to make any conclusions based on the anxiety levels of the animals. Additionally, some studies have indicated that A_1 antagonists are more anxiogenic profile than A_{2A} antagonists (Correa and Font, 2008; Florio et al., 1998). Thus it is possible the lack of motor activity found in the animals treated with SCH58261 may be due to a lack of anxiety, rather than an inability to move.

Until recently, it has been believed that all cases of Parkinson's disease were characterized by high levels of tremor and were thus often diagnosed via the presence of resting tremor in the extremities. However, examination of an early report by Hoehn and Yahr (1967) on the progression of PD shows that patients evidenced a marked diversity of symptoms, particularly with regard to tremor and akinesia. Similarly, other investigations have shown that there may be at least two distinct forms of PD: a form in which resting tremor is the dominant symptom and a form in which akinesia-rigidity is the dominant feature (Birkmayer et al., 1979; Jankovic et al., 1990; Korchounov et al., 2004; Schiess et al., 2000). Studies comparing these two sub-populations of PD patients have found evidence of different neurochemical and cellular profiles (Otsuka et al., 1996; Paulus and Jellinger, 1991; Vingerhoets et al., 1997). In 1991 Paulus and Jellinger extracted brains of deceased Parkinson's patients and found that those who were suffering from the akinetic-rigid form of Parkinson's showed greater neuron depletion in the substantia nigra at a consistent point of the progression of the disease, compared to patients who suffered from the tremor-dominant variant. An analysis of CSF by Schiess et al. (2000) found that akinetic-dominant patients had

higher concentrations of glycine and HVA and lower concentrations of 5-HIAA and 5-HT compared to tremor-dominant patients. Most recently Spiegel et al. (2007) found that akinetic-dominant patients had lower DA transporter binding than tremor-dominant patients. Another important and compelling distinction that has been made between the two subcategories is the rapid progression and more severe cognitive impairments and depressive symptoms associated more often with the akinetic form of Parkinson's disease as opposed to the tremor dominant form(Spiegel et al., 2007). Taken together these studies support the idea of different subtypes of PD. The dopaminergic signal from SNc to the striatum has been found to be critical for motor readiness and initiation (Hauber, 1998b). If this is in fact the case it would make sense that increased rigidity and lack of initiation would be seen in the akinetic form given an increased loss of dopamine. Given that adenosine receptors do not deteriorate in concordance with dopamine receptors (Hurley et al., 2000) and can in fact increase in number in response to dopamine depletion, it is not unreasonable to think that adenosine antagonism may be especially therapeutic in these cases.

The results of these studies indicate that adenosine antagonists may be promising therapies for PD, and that that A_{2A} antagonists may be more useful in patients with the akinetic-dominant form of PD. Although the role of A_1 receptors in PD is still unclear, these results suggest that antagonism of A_1 receptors may produce therapeutic effects, particularly at the beginning of treatment.

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