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Differential effects of adenosine antagonists in two models of parkinsonian tremor

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ABSTRACT

Adenosine A_1 and A_{2A} receptors are colocalized with dopamine D_1 and D_2 receptors on striatal projection neurons and adenosine antagonists have been proposed as adjunctive therapies to L-DOPA treatment in Parkinson patients. We present here two studies examining the effects of selective and non-selective adenosine antagonists in two rodent models of parkinsonian tremor. Tremulous jaw movements (TJMs) were induced by either the dopamine antagonist pimozide (1.0 mg/kg) or the acetylcholine agonist tacrine (5.0 mg/kg), and were quantified by a trained observer who was blind to the treatment conditions. Animals were treated concomitantly with either caffeine (10.0 mg/kg non-selective adenosine antagonist), 8-cyclopentyltheophylline (CPT; 10.0 mg/kg; selective A_1 antagonist) or SCH58261 (8.0 mg/kg; selective A_{2A} antagonist). Caffeine, CPT and SCH58261 all significantly reduced pimozide-induced TJM activity. Surprisingly administration of adenosine antagonists did not reduce tacrine-induced TJMs, and in the case of SCH58261 significantly increased TJMs compared to tacrine alone. These results indicate that antagonism at A_1 receptors may be more important for the reduction of tremor than previously supposed. Furthermore they indicate that dopamine antagonist-induced tremor models and acetylcholine agonist-induced tremor models are not entirely similar, and caution should be taken when using these models to evaluate novel therapeutics.

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons of the substantia nigra pars compacta (SNc; Blandini et al., 2000). As a result, normal dopaminergic modulation of the striatopallidal and striatonigral pathways is disrupted and basal ganglia (BG) function compromised; prominent symptoms include resting tremor, bradykinesia/akinesia, rigidity, and postural/gait disturbances (Colcher and Simuni, 1999). Clinical diagnosis is generally made upon presentation of either resting tremor or bradykinesia along with one of the other aforementioned symptoms and positive response to treatment with L-DOPA (Colcher and Simuni, 1999; Mayeux, 2003).

Traditional pharmacotherapy has focused on restoring dopamine (DA) levels with L-DOPA however its efficacy declines over time, requiring higher doses and increasing the likelihood of dyskinetic effects (Blandini et al., 2000; Julien 2005 p. 427). Furthermore, there is controversy over whether the metabolism of L-DOPA and/or DA *in vivo* accelerates SNc cell loss through oxidative stress (Clement et al., 2002; Simuni and Stern, 1999). As an alternative to traditional L-DOPA therapy, adenosine antagonists have gained attention as potential adjunctive compounds to help minimize the negative effects incurred by L-DOPA (Schwarzschild et al., 2006). The critical feature of adenosine antagonism lies in A_1–D_1 and A_{2A}–D_2 receptor co-localizations in striatonigral and striatopallidal neurons wherein adenosine and DA functionally oppose each other (Ferre et al., 1997, 2001). Evidence from biochemical studies has indicated that stimulation of striatal A_1 receptors antagonistically changes the binding characteristics of D_1 receptors (Ferre et al., 1994), and stimulation of striatal A_{2A} receptors decreases the affinity of D_2 receptors (Ferre et al., 1991b). In addition, D_1, D_2, A_1 and A_{2A} receptors are all coupled to adenylyl cyclase (AC); stimulation of either A_{2A} or D_1 receptors activates AC while stimulation of either A_1 or D_2 receptors decreases it (Fredholm, 1995; Gingrich and Caron, 1993). Thus, by targeting adenosinergic receptors, dopaminergic receptors are indirectly modulated as well. Particular interest has been paid to A_{2A} receptors because of their preferential expression in the striatopallidal pathway and their potential to regulate this pathway, which has been shown to be overactive in PD (Mori and Shindou, 2003; Wichmann and DeLong, 1996). As mentioned above, A_{2A} receptors and D_2 receptors act in an antagonistic manner; it is believed that a critical function of striatal dopamine is to antagonize tonically active signaling via A_{2A} receptors (Tanganelli et al., 2004; Vortherms and Watts, 2004). A loss of DA would lead to unopposed adenosine signaling (Fredholm and Svenningsson, 2003), resulting in overactivity of the striatopallidal pathway. In addition, the anatomical specificity of A_{2A} receptors provides an attractive opportunity for pharmaceutical agents to

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selectively target striatopallidal neurons (Xu et al., 2005). Behavioral studies using various selective A_{2A} antagonists such as KF 17837 (Correa et al., 2004), SCH58261 (Wardas et al., 2003), and KW 6002 (Bibbiani et al., 2003; Kanda et al., 2000; Shiozaki et al., 1999) have shown improvements of motor symptoms in both rodent and non-human primate models of PD. Furthermore, when KW 6002 (istradefylline) is coadministered with low dose L-DOPA, PD patients have experienced improvements in duration of antiparkinsonian activity as well as reductions in all cardinal signs of parkinsonism, particularly tremor (Bara-Jimenez et al., 2003; Chase et al., 2003).

The majority of research examining the effectiveness of adenosine antagonists in rodent models of PD symptoms has typically used gross motor behaviors such as catalepsy and hypolocomotion (Chartoff et al., 1999; Ferre et al., 1991a; Florio et al., 1997; Kanda et al., 1994; Marston et al., 1998; Nikodijevic et al., 1991; Popoli et al., 1996; Shiozaki et al., 1999; Snyder et al., 1981; Stasi et al., 2006; Villanueva-Toledo et al., 2003; Zarrindast et al., 1993) while only a handful of studies have investigated the effectiveness of adenosine antagonism for tremor (Correa et al., 2004; Simola et al., 2004; Simola et al., 2006). Tremulous jaw movements, defined as "rapid vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus" (Salamone et al., 1998) have been used as a rodent model of Parkinsonian tremor and are commonly induced by two different methods: DA antagonism or depletion and muscarinic agonism. Both methods have been well characterized (Betz et al., 2007; Correa et al., 2004; Finn et al., 1997; Ishiwari et al., 2005; Mayorga et al., 1997; Simola et al., 2004, 2006). In the striatum, DA and acetylcholine (ACh) functionally oppose each other such that a decrease in one is accompanied by a corresponding increase in the other (Cousins et al., 1999; Finn et al., 1997; Salamone and Baskin, 1996; Salamone et al., 1998). Although the exact mechanisms underlying this interaction have yet to be elucidated, it has been suggested that DA antagonism or depletion leads to increased ACh release in the striatum and that this increase is responsible for TJM induction (Cousins et al., 1999; Finn et al., 1997; Salamone and Baskin, 1996). Both methods induce tremors that share neuroanatomical, pharmacological and temporal characteristics. Regardless of whether DA antagonists or cholinomimetics are used, the critical site mediating TJM production has been shown to be the ventrolateral striatum (Cousins et al., 1999; Finn et al., 1997; Kelley et al., 1989; Mayorga et al., 1997). Previous research has also demonstrated that the temporal characteristics following either method are remarkably similar (Ishiwari et al., 2005; Salamone and Baskin, 1996). There are, however, some critical differences between the two models. The muscarinic agonism model generally induces a more robust total number of TJMs (5–6 fold higher) and the induction is fairly rapid (~10 min; Mayorga et al., 1997; Salamone and Baskin, 1996). On the other hand, the dopamine antagonism/depletion model generally induces fewer overall TJMs (though the bursting pattern and Hz rate are similar) and it takes longer to induce TJMs when using this model (~5–14 days; Egan et al., 1996; Glassman and Glassman, 1980; Jicha and Salamone, 1991; Steinpreis and Salamone, 1993; Steinpreis et al., 1993).

As noted above, only a few studies have examined the effects of adenosine antagonists on tremor, and the tremor models used in these studies have varied, with some investigators using the DA antagonism/depletion model (Correa et al., 2004) while others have used the ACh agonism model (Simola et al., 2004, 2006). The aim of the present study was to compare the effects of adenosine antagonists on tremor induced by either DA antagonism or ACh agonism. To more fully understand the relationship between DA, ACh and adenosine three adenosine antagonists were compared in each tremor model: the non-selective antagonist caffeine, the selective A_1 antagonist 8-cyclopentyltheophylline (CPT, K_i[nM] = 24, Bruns et al., 1986) and the selective A_{2A} antagonist SCH58261 (SCH, K_i[nM] = 0.70, Zocchi et al., 1996).

1. Methods

1.1. Experiment 1: Effects of caffeine, CPT, and SCH58261 on TJMs induced by the DA D_2 antagonist pimozide

1.1.1. Subjects

Fifty drug naïve male Sprague–Dawley rats (Simonsen Laboratories; Gilroy, CA, USA) weighing 260–280 g at the beginning of the experiment were used. Rats were group housed in plastic cages with pelleted bedding and had access to food and water *ad libitum*. The vivarium followed a 12 h light/dark cycle with lights on at 07:00 h and temperature maintained at approximately 23 °C. The animals were cared for and treated according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and the experimental protocol was approved by California State University's Institutional Animal Care and Use Committee (IACUC).

1.1.2. Drugs

Pimozide and CPT were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, US), SCH58261 was purchased from Tocris Bioscience (Ellisville, MO, US), and caffeine was purchased from MP Biomedicals (Solon, OH, US). Pimozide (1.0 mg/kg), SCH58261 (8.0 mg/kg) and caffeine (10.0 mg/kg) were dissolved in 0.3% tartaric acid which served as the vehicle control. CPT (10.0 mg/kg) was dissolved in 0.9% NaCl with 0.1 N NaOH. The doses of pimozide, SCH58261, CPT and caffeine were based on those from previous studies (Betz et al., 2007; Ishiwari et al., 2005; Simola et al., 2004).

1.1.3. Procedures

The procedures used in the present study for TJM induction were based upon previous studies (see Betz et al., 2007; Ishiwari et al., 2005). A total of 40 rats were given daily intraperitoneal (i.p.) injections of 1.0 mg/kg pimozide in a volume of 1.0 ml/kg for 8 days while the remaining 10 were given vehicle control. On day eight, 3 h and 40 min following pimozide or vehicle injections, pimozide treated rats received a second injection of either CPT (10.0 mg/kg, $n = 10$), SCH58261 (8.0 mg/kg, $n = 10$), or caffeine (10.0 mg/kg, $n = 10$). Vehicle treated rats received a second injection of vehicle. Ten minutes after the second injection each rat was placed in a Plexiglas box on a raised platform that allowed for viewing from all angles. After a 10 min habituation period, TJM activity was counted for a period of 5 min using a mechanical hand counter by a trained observer who was blind to the conditions. TJMs were defined as "rapid vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus" (see Salamone et al., 1998); each vertical deflection was counted as one TJM. When rats groomed themselves, a 5 s delay period after the last observed grooming behavior followed before counting recommenced to avoid possible confounds related to grooming.

1.1.4. Design and analysis

Day eight data was analyzed using an incomplete 2 (dopamine antagonist; pimozide or vehicle) × 4 (adenosine antagonist; caffeine, SCH58261, CPT or vehicle) factorial design (see Table 1). For the purposes of data analysis the two independent variables were collapsed into

Table 1
Experiment 1 treatment design.

DA antagonist	Adenosine antagonist			
	Vehicle	Caffeine 10.0 mg/kg	SCH 58261 8.0 mg/kg	CPT 10.0 mg/kg
Vehicle	$n = 10$ treatment 1 (control)			
Pimozide 1.0 mg/kg	$n = 10$ treatment 2 (model)	$n = 10$ treatment 3	$n = 10$ treatment 4	$n = 10$ treatment 5

a single variable, *treatment condition*, with 5 levels: vehicle + vehicle (veh/veh; treatment 1), pimozide + vehicle (pim/veh; treatment 2), pimozide + caffeine (pim/caff; treatment 3), pimozide + CPT (pim/CPT; treatment 4), pimozide + SCH58261 (pim/SCH; treatment 5). Data were analyzed using ANOVA procedures, followed by a priori Dunnett's comparisons to examine differences between treatment 2 (pim/veh) and the other groups. *t*-tests using a modified Bonferroni correction were used to determine which treatment conditions were significantly different from the veh/veh group (Keppel, 1982).

1.2. Experiment 2: Effects of caffeine, CPT, and SCH58261 on TJMs induced by the acetylcholinesterase tacrine

1.2.1. Subjects

Fifty drug naïve male Sprague–Dawley rats (Simonsen Laboratories; Gilroy, CA, USA) weighing 260–280 g at the beginning of the experiment were used. Rats were group housed and cared for as described in Experiment 1.

1.2.2. Drugs

Tacrine and CPT were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, US), SCH58261 was purchased from Tocris Bioscience (Ellisville, MO, US), and caffeine was purchased from MP Biomedicals (Solon, OH, US). Tacrine was dissolved in 0.9% NaCl which also served as the vehicle control. Caffeine, SCH58261, and CPT were dissolved as described in Experiment 1.

1.2.3. Procedures

As opposed to the subchronic treatment protocol for pimozide-induced TJMs, acute administration of tacrine is sufficient to induce TJMs (see Mayorga et al., 1997). Fifty rats were subdivided into five groups of 10 rats each and given an initial injection of adenosine antagonist [10.0 mg/kg CPT ($n=10$); 8.0 mg/kg SCH ($n=10$); or 10.0 mg/kg caffeine ($n=10$)] or vehicle control ($n=20$). Ten minutes after the initial injection, a second injection of 5.0 mg/kg tacrine was administered to the adenosine antagonist treated rats and 10 of the vehicle treated rats. The remaining 10 vehicle treated rats received a second injection of vehicle and served as the control group. Immediately following the second injection, rats were placed in the Plexiglas box as described above for a 10 min habituation period. Following habituation each rat was observed for a period of 5 min using the same TJM counting method as described in Experiment 1.

1.2.4. Design and analysis

Data was analyzed using an incomplete 2 (vehicle vs. tacrine) × 4 (adenosine antagonist; caffeine, SCH58261, CPT or vehicle) factorial design (see Table 2). As described in Experiment 1, the two independent variables were collapsed into one independent variable, *treatment condition*, with 5 levels: vehicle + vehicle (veh/veh; treatment 1), tacrine + vehicle (tac/veh; treatment 2), tacrine + caffeine (tac/caff; treatment 3), tacrine + CPT (tac/CPT; treatment 4), tacrine + SCH58261 (tac/SCH; treatment 5). Data were analyzed using ANOVA procedures, followed by a priori Dunnett's comparisons to examine differences between treatment 2 (tacrine + vehicle) and the other groups. *t*-tests using a modified Bonferroni correction were used to determine which treatment conditions were significantly different from the veh/veh group (Keppel, 1982).

Table 2
Experiment 2 treatment design.

Cholinesterase inhibitor	Adenosine antagonist			
	Vehicle	Caffeine 10.0 mg/kg	SCH 58261 8.0 mg/kg	CPT 10.0 mg/kg
Vehicle	$n=10$ treatment 1 (control)			
Tacrine 5.0 mg/kg	$n=10$ treatment 2 (model)	$n=10$ treatment 3	$n=10$ treatment 4	$n=10$ treatment 5

2. Results

2.1. Experiment 1: Effects of caffeine, CPT, and SCH58261 on pimozide-induced TJMs

Data screening procedures revealed four outlier scores (each in a different treatment condition: pim/veh, pim/caff, pim/CPT, and pim/SCH) in the Day 8 data (i.e. more than two standard deviations from the mean); these scores were subsequently omitted from further analysis. One-way ANOVA revealed a significant difference among treatment conditions, $F(4, 41)=4.069$, $p<0.01$ (see Fig. 1). A priori Dunnett's comparisons were used to evaluate the differences between treatment 2 (pim/veh; control) and the other groups. It was found that there were significantly more TJMs in the pim/veh condition ($M=22.00$, S.E.M. $=\pm 3.274$) compared to the veh/veh condition ($M=8.60$, S.E.M. $=\pm 2.045$), $p<0.01$. With regard to adenosine antagonist treatment, caffeine ($M=10.89$, S.E.M. $=\pm 2.600$; $p<0.01$), CPT ($M=8.89$, S.E.M. $=\pm 2.475$; $p<0.01$) and SCH58261 ($M=13.0$, S.E.M. $=\pm 3.145$; $p<0.05$) each significantly reduced TJMs compared to pimozide alone. Additionally, independent sample *t* tests comparing pim/caff treatment and pim/CPT treatment with the veh/veh condition were non-significant, $t(17)=0$ .699, $p=0.494$, and $t(17)=0.091$, $p=0.929$, respectively, indicating that both drugs restored the behavior to control levels.

2.2. Experiment 2: Effects of caffeine, CPT, and SCH58261 on tacrine-induced TJMs

Data screening procedures revealed a single outlier in the veh/veh condition (i.e. more than two standard deviations from the mean); this score was subsequently omitted from further analysis. One-way ANOVA revealed a significant difference among treatment conditions, $F(4, 44)=20.909$, $p<0.001$ (see Fig. 2). A priori Dunnett's comparisons were used to evaluate the differences between treatment 2 (tac/veh; control) and the other groups. As expected, rats in the tac/veh condition displayed significantly more TJMs ($M=139.70$, S.E.M. $=\pm 19.842$) than those in the veh/veh condition ($M=4.33$, S.E.M. $=\pm 1.014$), $p<0.01$. Surprisingly, the data indicated that adenosine antagonism had an overall exacerbating effect on tacrine-induced TJMs, with SCH58261 producing the most robust effect ($M=229.40$, S.E.M. $=27.738$), $p<0.01$. Although treatment with caffeine ($M=173.50$, S.E.M. $=\pm 13.340$) and CPT ($M=156.60$, S.E.M. $=13.108$) produced greater TJMs than tacrine alone, the effects were not statistically significant. However, tacrine-induced TJMs following either caffeine or CPT treatment remained significantly higher than control, $t(17)=11.967$, $p<0$ .001 and $t(17)=10.962$, $p<0.001$, indicating that neither drug reduced tacrine-induced TJMs.

3. Discussion

Animal models of PD have typically investigated the effects of various adenosine antagonists on reversing gross motor deficits such as hypolocomotion and catalepsy (Kanda et al., 1994; Kase et al., 2003; Mandhane et al., 1997; Marston et al., 1998; Popoli et al., 1996; Shiozaki et al., 1999) while less attention has been paid to the amelioration of fine motor complications such as tremor (Correa et al., 2004; Salamone et al., 2008; Simola et al., 2006). However, as it has been estimated that tremor occurs in 75% of PD patients (Colcher and Simuni, 1999) it is imperative that reliable models of tremor are established to facilitate the evaluation of potential therapeutic compounds.

The results of the present studies confirm that both pimozide and tacrine can be used to induce tremorogenic activity in rats. Pimozide

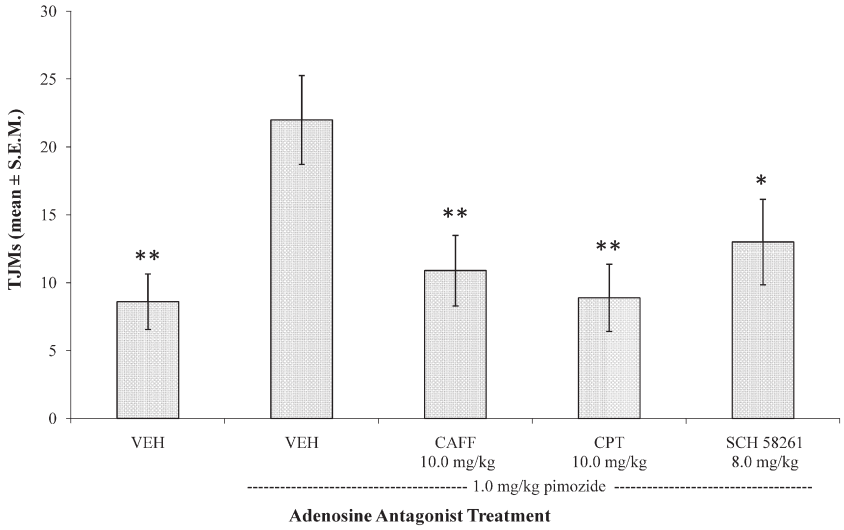

Fig. 1. Effects of selective (CPT and SCH 58261) and non-selective (caffeine) adenosine antagonists on pimozide-induced tremulous jaw movements (TJMs). Results shown as means ± standard error of measurement (S.E.M.). Treatment with adenosine antagonists significantly reduces pimozide-induced TJMs, $F(4, 41) = 4.069$, $p < .01$. *A priori* Dunnet's comparisons show significant differences between pim/veh group and veh/veh, pim/caff, pim/CPT and pim/SCH groups (*$p < .05$; **$p < .01$). pim/caff not significantly different from veh/veh, $t(17) = .699$, $p = .494$. pim/CPT not significantly different from veh/veh, $t(17) = .091$, $p = .929$.

acts as a D_2 receptor antagonist, and as discussed previously, striatal D_2 receptors are localized primarily on striatopallidal projection neurons. The activation of D_2 receptors is thought to inhibit the activity of striatal neurons, thus the current finding fits well within the theoretical framework of an overactive striatopallidal pathway resulting from loss of dopaminergic tone (Mori and Shindou, 2003; Wichmann and DeLong, 1996). The results from Experiment 2 coincide with past research demonstrating the more robust effects of muscarinic agonism over DA antagonism on TJM activity (Finn et al., 1997); it has been suggested that this may indicate a direct effect of muscarinic agonism and an indirect effect of DA antagonism on the production of TJMs. Furthermore, it has been shown that the muscarinic agonist, pilocarpine, had an additive effect on haloperidol-induced jaw movements (Rupniak et al., 1983).

The non-selective adenosine antagonist caffeine, the selective A_1 antagonist CPT, and the selective A_{2A} antagonist SCH58261 were able to significantly reduce pimozide-induced TJMs. These results agree with past findings and support the idea that adenosine antagonists can reverse dopamine antagonist- and dopamine depletion-induced tremor (Correa et al., 2004; Tronci et al., 2007). The present study used CPT, which is selective for the A_1 receptor (K_i[nM] = 24; 130-fold over A_{2A}); however it is not as selective for the A_1 receptor as other compounds such as DPCPX (K_i[nM] = 1.0; 500-fold over A2A; Abo-Salem et al., 2004; Bruns et al., 1986). Other investigators using similar paradigms have found A_1 antagonists to be ineffective at reversing DA-antagonist-induced behaviors (Mott et al., 2009; Salamone et al., 2009; Varty et al., 2008). The ability of CPT to reduce pimozide-induced TJMs in the present study might be due actions at A_{2A} receptors, particularly given the high dose of CPT (10.0 mg/kg) that was used. Future studies should investigate the ability of CPT to reduce pimozide-induced TJMs at lower doses. Still, it was interesting to note that the non-selective antagonist caffeine and the selective A_1 antagonist CPT reduced TJMs to a level that was not different from the veh/veh group, while the selective A_{2A} antagonist SCH58261 produced a less robust reduction. This was somewhat surprising as striatal A_{2A} receptors have been found to be principally located on

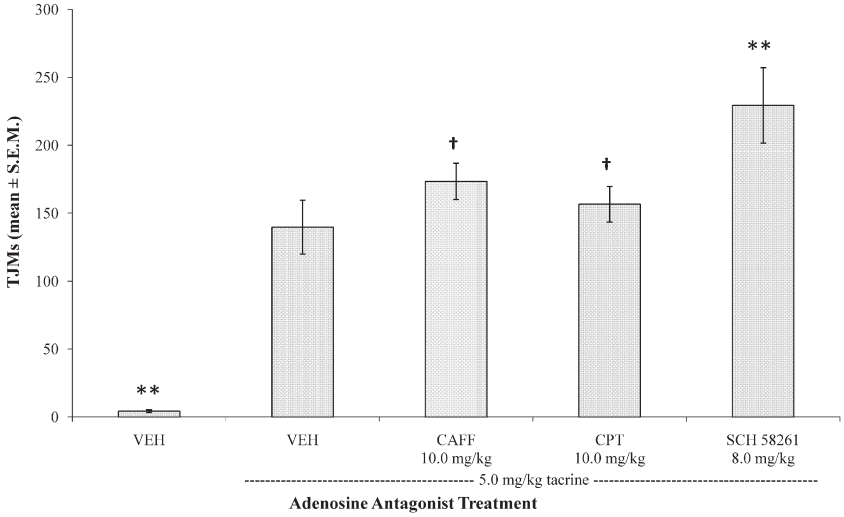

Fig. 2. Effects of selective (CPT and SCH 58261) and non-selective (caffeine) adenosine antagonists on tacrine-induced tremulous jaw movements (TJMs). Results shown as means ± standard errors of measurement (S.E.M.). Treatment with adenosine antagonists significantly affects tacrine-induced TJMs, $F(4, 44) = 20.909$, $p < .001$. *A priori* Dunnet's comparisons show significant differences between Tac/Veh group, veh/veh and Tac/SCH groups (**$p < .01$). †Caff/tac significantly different from veh/veh, $t(17) = 11.967$, $p < .001$. †CPT/tac significantly different from veh/veh, $t(17) = 10.962$, $p < .001$.

striatopallidal neurons (Mori and Shindou, 2003; Rosin et al., 2003; Schiffmann et al., 2007) which have been shown to be overactive in response to dopamine depletion (Mori and Shindou, 2003; Wichmann and DeLong, 1996). However, it has been proposed that while both A_1 and A_{2A} antagonism produce motor stimulatory effects, A_1 antagonism may play a greater role when administered acutely while A_{2A} antagonism makes a greater contribution following chronic administration due to the development of tolerance at A_1 receptors (Antoniou et al., 2005; Karcz-Kubicha et al., 2003; Quarta et al., 2004). The findings of the present study support the theory that A_1 receptors may have a stronger influence in acute paradigms, particularly at high doses. As this series of experiment was intended to be exploratory in nature only a single dose of each adenosine antagonist was used; while this does limit the conclusions that can be drawn from these studies, it should be noted that other studies conducted in this laboratory examining the acute effects of CPT (2.0–10.0 mg) and SCH58261 (2.5–10.0 mg) on haloperidol-induced hypolocomotion showed a similar pattern of results, with CPT significantly increasing locomotor activity while SCH58261 did not (Trevitt et al., 2009).

The effects of adenosine antagonists on tacrine-induced TJMs were quite surprising (see Fig. 2); they appeared to exacerbate the effects of tacrine. These results are contrary to previous studies using similar protocols (Simola et al., 2004) and may challenge the current understanding of adenosine–ACh interactions. In the 2004 study by Simola et al., the authors examined the ability of three doses of SCH58261 (2.0, 5.0 and 10.0 mg/kg) to reduce TJMs induced by 2.5 mg/kg tacrine. It was found that although the 2.0 mg/kg did not significantly reduce TJMs, both the 5.0 mg/kg and 10.0 mg/kg doses did. Although the authors did not include the highest dose of SCH58261 (10.0 mg/kg) in their graphs, it appears as though the 5.0 mg/kg dose was the most effective at reducing TJMs, while the 10 m/kg dose was less effective (Simola et al., 2004). This may indicate that the effect of SCH58261 on tacrine-induced TJMs is not linear; it may be that higher doses of SCH58261 become less effective at reducing tacrine-induced TJMs. Indeed, while some studies examining the cellular response to adenosine antagonists have shown that adenosine antagonism decreases ACh release (Kurokawa et al., 1994), other studies have indicated that adenosine antagonists *increase* ACh release (Carter et al., 1995; Dunwiddie and Masino, 2001). The present study not only used a higher dose of SCH58261 (8.0 mg/kg) but it also used a higher dose of tacrine to induce TJMs (5.0 mg/kg). It may be that given a relatively high dose of tacrine the addition of an adenosine antagonist would only serve to increase ACh release; it is possible that the effect of adenosine antagonist administration was to further increase ACh levels, resulting in increased TJMs. Additionally, a pilot study conducted in our laboratory using 2.5 mg/kg tacrine and caffeine (5.0–20.0 mg/kg) found similar results; increasing doses of caffeine lead to exacerbation of the TJMs induced by 2.5 mg/kg tacrine (data not shown). Further testing using different dose combinations of tacrine and SCH58261 will be necessary to more fully describe this relationship.

An important issue these findings bring up is a possible problem with equating the tacrine-induced TJM model and the DA antagonist-induced TJM model. Much research has been done examining both models and comparing them on pharmacological, biochemical and temporal indices (see Salamone et al., 1998) and while the models appear to be quite similar, there are some differences. Cholinomimetics usually induce a higher frequency of TJMs compared to DA antagonists; generally speaking 5–6 times higher (Wisniecki et al., 2003). In addition, it is possible to induce a robust level of TJMs with acute administration of cholinomimentics; it usually takes between 5 and 14 days to induce an adequate level of TJMs using DA antagonists. It has also been shown that tacrine-induced TJMs respond differently than haloperidol-induced TJMs to treatment with GABA antagonists (Wisniecki et al., 2003). The results of the present study further indicate that under some conditions cholinomimetic-induced and DA-antagonist-induced TJMs may not be equivalent. This is especially important given that these models are being used to evaluate novel therapeutics (Salamone et al., 2008). Currently much is known about the relationship between DA and ACh in the striatum, as well as DA and adenosine. However, not much is known about the relationship between ACh, adenosine and DA together.

Given the discrepancies in the literature, future research should investigate the interrelationships between DA, ACh and adenosine at differing concentrations. Although much of the current direction in the development of novel pharmacological compounds for the treatment of PD has focused on A_{2A} antagonists, clarification of the dose–response parameters of A_{2A} receptor blockade may indicate limitations in this approach. Additionally, further examination of the roles of A_1 and A_{2A} receptor subtypes may shift the spotlight to include A_1 antagonists. Compounds with greater selectivity for both receptor subtypes and increased potency over caffeine may demonstrate clinical efficacy beyond that of A_{2A} antagonism alone. Indeed, it has been suggested that A_{2A} receptors are "necessary, but not sufficient" for motor activating effects of caffeine (Karcz-Kubicha et al., 2003). Additionally Jacobson et al. (1993) showed an increase in effectiveness of combined A_1 and A_{2A} antagonist treatment than either compound alone. Although both methods for TJM induction seem interchangeable in many respects, greater clarification of the interactions among DA, ACh, and adenosine will help direct future drug development.

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