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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