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Research Report

Deletion of adenosine A_1 or A_{2A} receptors reduces L-3,4-dihydroxyphenylalanine-induced dyskinesia in a model of Parkinson's disease

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ABSTRACT

Adenosine A_{2A} receptor antagonism provides a promising approach to developing nondopaminergic therapy for Parkinson's disease (PD). Clinical trials of A_{2A} antagonists have targeted PD patients with L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) in an effort to improve parkinsonian symptoms. The role of adenosine in the development of LID is little known, especially regarding its actions via A_1 receptors. We aimed to examine the effects of genetic deletion and pharmacological blockade of A_1 and/or A_{2A} receptors on the development of LID, on the induction of molecular markers of LID including striatal preprodynorphin and preproenkephalin (PPE), and on the integrity of dopaminergic nigrostriatal neurons in hemiparkinsonian mice. Following a unilateral 6-hydroxydopamine lesion A_1, A_{2A} and double A_1–A_{2A} knockout (KO) and wild-type littermate mice, and mice pretreated with caffeine (an antagonist of both A_1 and A_{2A} receptors) or saline were treated daily for 18–21 days with a low dose of L-DOPA. Total abnormal involuntary movements (AIMs, a measure of LID) were significantly attenuated ($p<0.05$) in A_1 and A_{2A} KOs, but not in A_1–A_{2A} KOs and caffeine-pretreated mice. An elevation of PPE mRNA ipsilateral to the lesion in WT mice was reduced in all KO mice. In addition, neuronal integrity assessed by striatal dopamine content was similar in all KOs and caffeine-pretreated mice following 6-hydroxydopamine lesioning. Our findings raise the possibility that A_1 or A_{2A} receptors blockade might also confer a disease-modifying benefit of reduced *risk* of disabling LID, whereas the effect of their combined inactivation is less clear.

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Abbreviations: AIM, abnormal involuntary movement; KO, knockout; L-DOPA, L-3,4-dihydroxyphenylalanine; 6-OHDA, 6-hydroxydopamine; LID, L-DOPA-induced dyskinesia; PD, Parkinson's disease; PPD, preprodynorphin; PPE, preproenkephalin; WT, wild-type

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1. Introduction

Blockade of adenosine A_{2A} receptors is being pursued as a non-dopaminergic alternative or adjunctive treatment of Parkinson's disease (PD). Several studies have investigated the usefulness of A_{2A} receptor antagonism to treat L-DOPA-induced dyskinesia (LID), a complication from current PD therapy, in both animal models and clinical trials (Chen, 2003; Morelli et al., 2007). The use of A_{2A} antagonists for symptomatic benefit with reduced risk of adverse effects in PD and LID is based *inter alia* on the discrete CNS distribution of the A_{2A} receptor and its colocalization with D_2 receptors in the "indirect" pathway of the basal ganglia motor circuitry (Ferre et al., 1997; Kase, 2001; Fredholm et al., 2003). Elimination or blockade of A_{2A} receptors expressed by forebrain neurons attenuates LID and related behaviors in hemiparkinsonian rodents or parkinsonian non-human primates (Bibbiani et al., 2003; Xiao et al., 2006).

Adenosine also activates the adenosine A_1 receptor, which–in contrast to discretely expressed A_{2A} receptor–is widely distributed throughout the CNS including the hippocampus and cortex as well as on the striatal neurons of the "direct" and "indirect" pathways of the basal ganglia (Fastbom et al., 1987; Ferre et al., 1996; Johansson et al., 1997; Tohyama and Takatsuji, 1998) making a selective action difficult to deduce. It has been proposed that blocking A_1 receptors on striatonigral neurons of the direct pathway may facilitate motor activity by disinhibiting the motor stimulant actions of colocalized dopamine D_1 receptors on these neurons, whereas blocking A_{2A} receptors on striatopallidal neurons of the indirect pathway may produce a parallel behavioral activation by mimicking the motor stimulant actions of colocalized D_2 receptors on these neurons (Ferre et al., 1997). By contrast, presynaptic A_1 and A_{2A} receptors (e.g., those colocalized on corticostriatal nerve terminals) can inhibit and activate, respectively, adenylyl cyclase and thus transmitter release (van Calker et al., 1979; Olah and Stiles, 1995; Dunwiddie and Masino, 2001; Fredholm et al., 2005; Ciruela et al., 2006).

Thus adenosine may modulate LID through cooperative or opposing actions on two of its receptors in the CNS. To explore the roles of these receptors alone and in combination in a mouse model of LID in PD, we characterized single A_1 and A_{2A} knockout (KO), as well as double A_1–A_{2A} receptor KO phenotypes in (6-hydroxydopamine-lesioned) hemiparkinsonian mice treated daily with L-DOPA for three weeks. To avoid genetic background confounds mice were generated from double heterozygote crosses in a congenic C57Bl/6 background. In addition to this genetic approach to addressing adenosine receptor involvement in LID, we investigated the effect of pharmacological blockade of adenosine receptors using the widely consumed non-specific adenosine antagonist caffeine. The investigation of caffeine was prompted by preliminary clinical data that raised the possibility of a link between higher levels of caffeine consumption among PD patients and a reduced risk of subsequent dyskinesia development (Schwarzschild et al., 2003). We chose both a dose of caffeine, which elicits hyperlocomotion (15 mg/kg), and a lower dose of caffeine (3 mg/kg), which is capable of modifying neuroplasticity (as in that of conditioning preference) without necessarily producing a motor stimulant effect (Fredholm et al., 1999).

2. Results

2.1. *Effect of adenosine receptor knockout on 6-OHDA-induced neurotoxicity*

Previous studies showed that inactivation of A_{2A} receptors by either genetic depletion or pharmacological blockade (caffeine and more specific A_{2A} antagonists, but not a specific A_1 receptor antagonist) can protect against brain dopaminergic neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Chen et al., 2001a,b). Accordingly, we first determined whether the dopaminergic lesion induced by 6-OHDA differs between control mice and KO or caffeine pretreated mice. We found that genotype (Table 1) or pharmacological (Table 2) blockade did not affect levels of dopamine and its metabolite DOPAC in the 6-OHDA-lesioned and contralateral (non-lesioned) striata.

2.2. *Effect of adenosine receptor knockout on behavioral sensitization*

Following daily L-DOPA treatment, the hemiparkinsonian mice developed behavioral sensitization, as recorded by contralateral rotations and dyskinesia, quantified by an abnormal involuntary movements (AIMs) scale (Fig. 1). Acutely (on day 1), responses to L-DOPA were indistinguishable between adenosine receptor genotypes. Chronically, rotational sensitization on the plateau phase (days 11–21) in A_1 KO, A_{2A} KO or A_1–A_{2A} double KO mice showed a trend of attenuation over the 21 days, but the difference was not statistically significant ($p=0.12$, repeated measures model) in comparison

Table 1 – Neurochemical measure of nigrostriatal innervation in wild-type and adenosine receptor KO mice chronically treated with L-DOPA after a unilateral 6-OHDA lesion.

Genotype	DA (pm/mg tissue)	DOPAC (pm/mg tissue)
WT ($n=12$)		
Ipsilateral (lesioned)	4.2±1.4*	0.0±0.0*
Contralateral (intact)	143.6±7.5	11.3±1.7
A_{2A} KO ($n=9$)		
Ipsilateral (lesioned)	4.3±3.0*	0.7±0.5*
Contralateral (intact)	129±10	15.9±7.4
A_1 KO ($n=8$)		
Ipsilateral (lesioned)	6.9±3.0*	0.4±0.4*
Contralateral (intact)	120±14	9.7±0.9
A_1–A_{2A} KO ($n=7$)		
Ipsilateral (lesioned)	10.4±8.2*	1.2±0.5*
Contralateral (intact)	118±18	9.6±0.9

*$p<0.05$ versus respective contralateral (intact) striatum. The dopamine levels in the intact (right) side were not significantly different between wild-type and each of the three KO genotypes ($p>0.05$, Student's *t*-test).