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ORIGINAL ARTICLE

A Critical Evaluation of Behavioral Rodent Models of Motor Impairment Used for Screening of Antiparkinsonian Activity: The Case of Adenosine A_{2A} Receptor Antagonists

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Abstract Animal models of motor dysfunction constitute the basis for the screening of new drugs with potential efficacy in diseases characterized by motor impairment, such as Parkinson's Disease (PD). Taking adenosine A_{2A} receptor antagonists as an example of a new class of drugs for PD, the review will examine the most utilized rodent models of motor impairment and the results reported in the literature with this class of drugs. The results obtained so far in rodent models of PD suggested that A_{2A} receptor antagonists might have symptomatic therapeutic efficacy in PD. They may ameliorate initiation of movement, gait and muscle rigidity, sensorimotor integration deficits, and tremor. Moreover, A_{2A} receptor antagonists when administered with a low sub-threshold dose of L-DOPA potentiated its efficacy. However, the clinical trials so far performed have evaluated their efficacy in the "ON/OFF" of PD patients with motor complications, showing a limited efficacy of this class of drug. Therefore, on one hand, animal models of PD might have a limited validity; on the other hand, clinical trials should explore the efficacy of A_{2A} receptor antagonists on a broader range of parkinsonian conditions.

Keywords Parkinson's disease · Rodent models · Catalepsy · 6-Hydroxydopamine lesion · A_{2A} receptor antagonists · Adenosine

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Introduction

Parkinson's disease (PD) is a chronic neurological basal ganglia-associated disorder caused by progressive degeneration of the nigrostriatal dopaminergic neurons and is characterized by bradykinesia, rigidity, muscular stiffness, tremor, poor posture and balance, and sensorimotor integration deficits (Marsden 1994; Obeso et al. 2000). The disease still awaits suitable treatments since all the currently used drugs produce several side effects, including "ON/OFF" and "wearing-off" phenomena and dyskinesia (which is the most disabling) (Olanow 2004).

Although PD is one of many human diseases which do not appear to have spontaneously arisen in animals, the characteristic features of this disease can, however, be more or less accurately reproduced in animals through the administration of various neurotoxic agents or drugs disrupting dopaminergic neurotransmission. These animal models of motor dysfunction constitute the basis for the screening of new drugs with potential efficacy in diseases characterized by basal ganglia-related motor impairment, such as PD.

Since the first use of reserpine in rodents as a model of motor impairment (Horst et al. 1973; Duvoisin and Marsden 1974) several other models, based on drugs such as reserpine or on toxins have been developed. These models usually provide the first steps to taking the new compounds to clinical trials. This review will examine the most utilized rodent models of motor impairment that characterize PD and the results obtained with adenosine A_{2A} receptor antagonists in these models, starting from the first demonstration of their anticataleptic efficacy (Kanda et al. 1994) to the results in unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats (Pinna et al. 2010).

Table 1 Summary of the effects exerted by A_{2A} receptor antagonists on parkinsonian-like symptoms as compared with L-DOPA

Rodent model of PD	A_{2A} antagonists	L-DOPA
Antagonism of akinesia/catalepsy induced by reserpine or haloperidol	++	+++
Potentiation of L-DOPA on reversing catalepsy induced by reserpine or haloperidol	+++	
Attenuation of muscular rigidity produced by reserpine or haloperidol	++	+++
Potentiation of L-DOPA on reducing muscular rigidity produced by reserpine or haloperidol	+++	
Antagonism of tremulous jaw movements elicited by tremorigenic drugs	+++	+++
Induction of turning behavior in unilaterally 6-OHDA-lesioned rats	–	+++
Amplification of turning behavior elicited by dopaminergic drugs in unilaterally 6-OHDA-lesioned rats	+++	
Reversal of motor initiation deficit, forelimb akinesia/hypokinesia and sensorimotor integration deficit in unilaterally 6-OHDA-lesioned rats	++	+++
Reversal of the progressive shortening of the duration of contralateral turning during chronic treatment with L-DOPA in unilaterally 6-OHDA rats	+++	

The number of plus represents the intensity of the response. For details see text

Reserpine Model of PD

Administration of the monoamine-depleting agent reserpine in rodents was one of the first animal models utilized in PD research. Indeed, this model was fundamental in producing the first evidence of therapeutic efficacy of the gold-standard treatment for PD, L-3,4-dihydroxyphenylalanine (L-DOPA) (Carlsson et al. 1957) and provided important contributions to our understanding of the relationship between monoamine depletion and parkinsonian symptoms. In rodents, the alkaloid reserpine induces a dramatic reduction of motor activity, characterized principally by akinesia and hypokinesia, which are representative of parkinsonian symptoms (Gerlach and Riederer 1996; Duty and Jenner 2011). However, rodents treated with reserpine showed other parkinsonian-like symptoms, such as catalepsy, hindlimb rigidity, and tremor (as described in the following sections of the review) (Gerlach and Riederer 1996; Lorenc-Koci et al. 1996; Salamone et al. 2008; Duty and Jenner 2011).

Reserpine inhibits the vesicular monoamine transporter, VMAT2, leading to complete depletion of cerebral (and peripheral) monoamines, including serotonin, noradrenaline, and dopamine (Duty and Jenner 2011). Although, the PD model of reserpine has several limitations, including lack of selectivity for cerebral dopamine and lack of nigral dopaminergic cell degeneration, this model was very useful in predicting the efficacy of both dopaminergic and non-dopaminergic drugs that are then examined in more complex PD animal models. Indeed, all of the dopaminergic drugs in current clinical therapy of PD showed efficacy in reserpine-treated rodents, supporting the predictive validity of this model (for review see Duty and Jenner 2011).

In mice and rats, reserpine produced a marked decrease in horizontal and vertical locomotor activity and this effect, which reflects the depletion of striatal dopamine, is maintained for up to 24 h. A_{2A} antagonists such as KF17837 and KW-6002 (istradefylline) produced a dose-dependent reversal of hypolocomotion and/or catalepsy induced by reserpine in rats and/or mice (Kanda et al. 1994; Shiozaki et al. 1999) (Table 1). Similarly, new derivative adenosine A_{2A} and mixed A_{2A}/A_1 antagonists are active in reversing akinesia induced by reserpine in rodents (Drabczyńska et al. 2011; Shook et al. 2010, 2012) (Table 1). Moreover, in reserpine-treated rodents, administration of KF17837 or KW-6002 potentiated the anti-cataleptic effects of a low dose of L-DOPA, suggesting that there may be a synergism between the adenosine A_{2A} receptor antagonists and the dopaminergic agents (Kanda et al. 1994; Shiozaki et al. 1999) (Table 1).

Catalepsy and Muscle Rigidity Models of PD

Catalepsy is a behavioral state induced in rodents by administration of different pharmacological agents, such as typical neuroleptics, reserpine, or cholinomimetics, characterized by akinesia and muscular rigidity caused by hypofunctionality of the striatum (Gerlach and Riederer 1996). Drugs commonly used in PD treatment are known to counteract catalepsy (Elazar et al. 1990; Kobayashi et al. 1997). In particular, catalepsy induced by the dopamine receptor antagonist haloperidol is the most common chemical model of PD used to screen antiparkinsonian drugs. Thus, the majority of adenosine A_{2A} receptor antagonists have been evaluated in this experimental paradigm in order to confirm that binding to adenosine A_{2A} receptors results in functional antagonistic actions on this receptor (Pinna et al. 2005; Stasi et al. 2006; Neustadt et al. 2007; Gillespie et al. 2009; Shook et al. 2010) (Fig. 1; Table 1). Moreover, the catalepsy test is useful to underline the pharmacokinetic differences of the

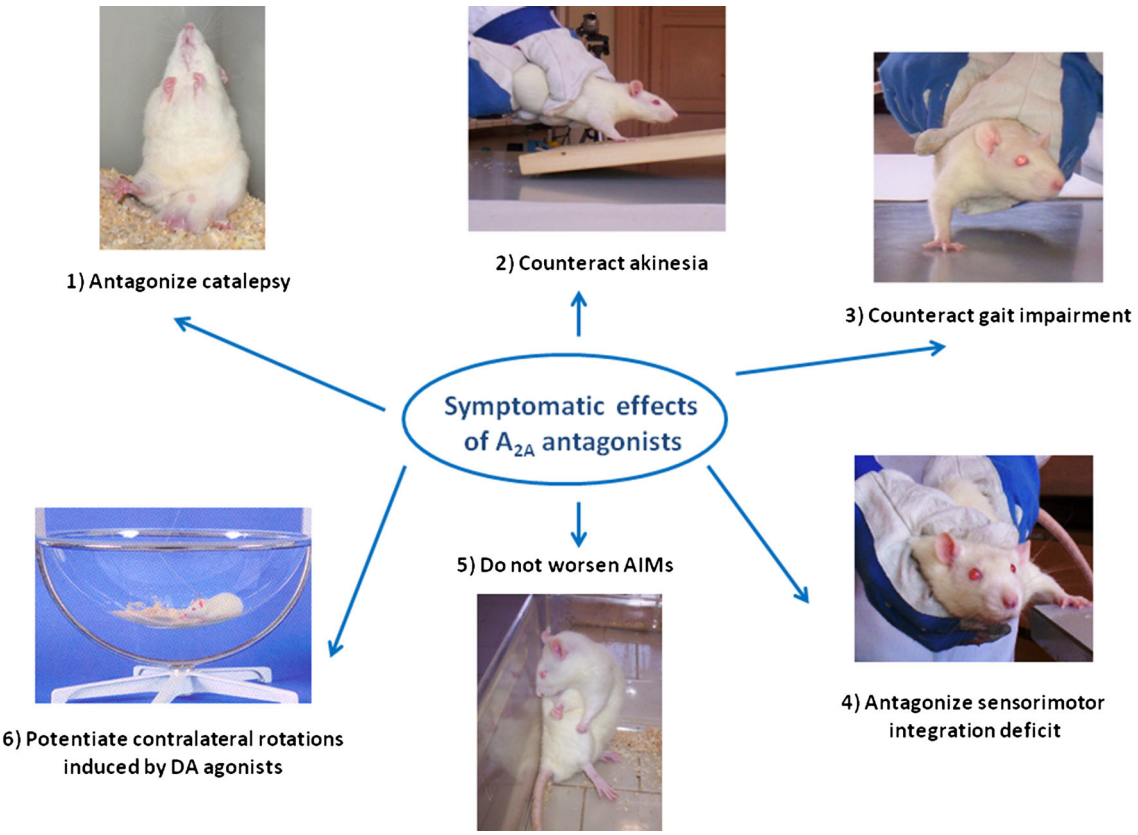

Fig. 1 Picture illustrating different rodent models utilized for behavioral evaluation of A_{2A} receptor antagonists. Clockwise shows test for *1* catalepsy, *2* akinesia, *3* gait impairment, *4* sensorimotor integration deficit, *5* abnormal involuntary movements (AIMs), and *6* contralateral rotation induced by dopamine (DA) agonists

compounds tested (Weiss et al. 2003; Pinna et al. 2005; Neustadt et al. 2007; Gillespie et al. 2009).

In the screening studies of new adenosine A_{2A} antagonists to assess their anti-cataleptic efficacy, 8-substituted 9-ethyladenine derivatives (ANR82, ANR94, and ANR152), were administered 90 min after haloperidol, in order to evaluate their effects on deeply cataleptic rats. All three compounds investigated reversed haloperidol-induced catalepsy, with some differences on both onset and duration of effect among the derivatives (Pinna et al. 2005). Consistent with these findings, numerous A_{2A} receptor antagonists, such as KF17837, DMPX, KW-6002, ZM241385, VER6947, VER7835, ST1535, SCH58261, SCH412348, SCH420814 (preladenant), and BIIB014 (vipadenant), were able to counteract catalepsy in rodents, reducing its duration and severity; therefore, demonstrating an improvement of parkinsonian motor impairment by these drugs (Kanda et al. 1994; Mandhane et al. 1997; Shiozaki et al. 1999; Villanueva-Toledo et al. 2003; Weiss et al. 2003; Stasi et al. 2006; Gillespie et al. 2009; Hodgson et al. 2009) (Table 1). Furthermore, the co-administration of several A_{2A} antagonists, such as KW-6002, KF17837, and ST1535, with L-DOPA has been demonstrated to strengthen the anti-cataleptic effect induced by L-DOPA, indicating the existence of a synergistic interaction between L-DOPA and A_{2A} antagonists (Kanda et al. 1994; Shiozaki et al. 1999; Stasi et al. 2006) (Table 1).

Another cardinal symptom manifested by parkinsonian patients, which can be as disabling as bradykinesia and akinesia, is muscle rigidity. Clinical muscular rigidity is characterized by an increased resistance to passive movement and could be mimicked in rodents by administration of adequate doses of haloperidol or reserpine; indeed, these two drugs induced muscle rigidity with many mechanographic and electromyographic features similar to those observed in PD patients (Lorenc-Koci et al. 1996). Both effects are reversed by blockade of the A_{2A} receptors with SCH58261, suggesting the existence of a beneficial effect of A_{2A} antagonists on parkinsonian-like muscular rigidity (Wardas et al. 2001; Wardas 2003) (Table 1). Moreover, the combination of SCH58261 with a dose of L-DOPA, which alone did not affect the haloperidol or reserpine-induced muscle rigidity, induced a pronounced synergistic effect by alleviating this symptom (Wardas et al. 2001; Wardas 2003) (Table 1). These beneficial effects on parkinsonian-like muscular rigidity of A_{2A} antagonists have been proposed to

be mediated by the facilitation of dopamine transmission at the postsynaptic level (Wardas et al. 2001; Wardas 2003).

Taken together, the efficacy of A_{2A} antagonists obtained in catalepsy and muscle rigidity in rodent models indicate that these drugs might be effective in specifically counteracting parkinsonian-like muscle rigidity, which is often resistant to common antiparkinsonian drugs.

Tremor Model of PD

Experimental models of parkinsonian tremor characterized by tremulous jaw movements (TJMs) induced by several pharmacological agents, including the muscarinic agonist pilocarpine, the acetylcholinesterase inhibitor tacrine, haloperidol, and the neurotoxin 6-OHDA, have been validated for evaluating the anti-tremorigenic effects of drugs (Salamone et al. 1998). TJMs induced by tremorigenic drugs show many of the electromyographic and pharmacological characteristics of parkinsonian tremor in humans. In fact, TJMs display a peak frequency in the 3–7 Hz range, which is similar to the frequency range reported for parkinsonian tremor (Collins-Praino et al. 2011). The validation of these parkinsonian models has been confirmed by the fact that TJMs induced by tremorigenic drugs can be attenuated by antiparkinsonian drugs, including L-DOPA, apomorphine, bromocriptine, amantadine, benztropine, pergolide, and ropinirole (Cousins et al. 1997; Mayorga et al. 1997; Salamone et al. 1998; Ishiwari et al. 2005).

Using the tremor model of tacrine, vertical deflections of the lower jaw not directed at a particular stimulus and burst of TJMs were counteracted by acute SCH58261, ST1535, or ANR94 (Simola et al. 2004, 2006; Tronci et al. 2007; Pinna et al. 2010) (Table 1). Moreover, acute SCH58261 and MSX-3 significantly reduced TJM after administration of a low dose of pilocarpine (Simola et al. 2006; Collins et al. 2010) (Table 1). Consistent with these results, acute administration of several A_{2A} antagonists KF17837, KW-6002, MSX-3, and the new pro-drug LUAA47070 significantly reversed jaw tremor stimulated by haloperidol, reserpine, and the dopamine antagonist pimozide in rats, suggesting a beneficial use of these compounds as specific drugs against this parkinsonian symptom (Correa et al. 2004; Salamone et al. 2008; Betz et al. 2009; Collins-Praino et al. 2011; Collins et al. 2012) (Table 1).

This anti-tremorigenic effect of A_{2A} antagonists has been localized, by infusion of the A_{2A} antagonist SCHBT2 (water-soluble analog of SCH58261), in different regions of the striatum, providing evidence of the critical role of the ventrolateral portion compared with the dorsolateral portion of striatum in completely reversing TJMs induced by tacrine (Simola et al. 2004). Notably, a specific increase in adenosine A_{2A} receptor mRNA expression in this striatal region was detected following dopamine denervation in 6-OHDA-lesioned rats (Pinna et al. 2002). On the basis of the critical role exerted by the increase in striatal acetylcholine in promoting the genesis of TJMs (Salamone et al. 1998), it has been hypothesized that modulation of cholinergic transmission by A_{2A} antagonists might mainly underlie the anti-tremorigenic effects of these drugs observed in rats. Indeed, these interactions between A_{2A} antagonists and acetylcholine have been described to occur mainly at the presynaptic level where according to some studies (Kurokawa et al. 1994), blockade of A_{2A} receptors located on striatal cholinergic interneurons can reduce the evoked release of acetylcholine. However, this mechanism is still poorly understood and others studies did not find the same type of interaction (Jin and Fredholm 1997).

Overall, these finding of the effectiveness of A_{2A} antagonists obtained in rodent models of parkinsonian tremor indicate that blockade of A_{2A} receptors might help to reduce parkinsonian-like resting tremor, a symptom very difficult to counteract.

Effects of A_{2A} Antagonists on Behavioral Models of PD of Unilateral 6-OHDA-Lesioned (Hemiparkinsonian) Rats

The most frequently used rodent PD model is characterized by intracerebral infusion of the dopaminergic neurotoxin 6-OHDA (Ungerstedt 1968). This neurotoxin induces a massive degeneration of nigrostriatal dopaminergic neurons, mimicking that occurring in idiopathic PD and resulting in the presence of parkinsonian-like symptoms (akinesia, bradykinesia, sensorimotor integration deficits, etc.) in the side contralateral to the lesion, thus providing a valuable PD model (Schwarting and Huston 1996; Simola et al. 2007; Duty and Jenner 2011). Specifically, unilateral injection of 6-OHDA in the medial forebrain bundle, the substantia nigra pars compacta (SNc), or the striatum causes a functional imbalance between the two striata, leading administration of dopaminergic drugs to elicit an unilateral motor impairment which is typically expressed by spontaneous ipsilateral rotational ("turning") behavior. Administration of the dopamine precursor L-DOPA or drugs directly stimulating dopamine receptors, results in a dose-dependent contralateral rotational behavior due to the supersensitivity of striatal dopamine receptors, which develops as a consequence of dopamine depletion (Pinna et al. 1996; Schwarting and Huston 1996; Fenu et al. 1997). Rotation intensity is directly correlated with the dopaminergic nigrostriatal degeneration and with the dose of the drug administered, offering a valuable "in vivo" measure of drug efficacy and SNc dopaminergic neuron degeneration. In this model, the ability of a specific drug to induce

contralateral rotational behavior, as well as to potentiate the rotational behavior stimulated by dopamine receptor agonists, can be assumed as a parameter reflecting its antiparkinsonian activity (Schwarting and Huston 1996; Simola et al. 2007; Duty and Jenner 2011).

Blockade of A_{2A} receptors produced a motor facilitative activity in this rodent PD model. Specifically, in unilateral 6-OHDA-lesioned (hemiparkinsonian) rats, acute administration of different adenosine A_{2A} receptor antagonists, such as KW-6002, SCH58261, ANR94, ANR152, KF17837, VER6947, ST1535, SCH412348, and SCH420814, induced no contralateral rotations per se, but significantly potentiated rotational behavior induced by L-DOPA or apomorphine and by either D_1 or D_2 dopamine receptor agonists (Vellucci et al. 1993; Pollack and Fink 1996; Pinna et al. 1996, 2005, 2010; Fenu et al. 1997; Koga et al. 2000; Weiss et al. 2003; Rose et al. 2007; Tronci et al. 2007; Hodgson et al. 2009) (Fig. 1; Table 1). Moreover, administration of ANR94 and ANR152 produced significant contralateral rotational behavior in rats sensitized with L-DOPA (Pinna et al. 2005); whereas rats with a single injection of L-DOPA (not sensitized) did not rotate in response to ANR94 or ANR152 administered singularly (Pinna et al. 2005).

Stepping Test

In addition to the rotational behavior response, more fine features of PD symptomatology have been investigated using the 6-OHDA rat model. As a consequence of 6-OHDA lesion, rats develop forelimb akinesia, gait impairment, and sensorimotor integration deficits considered analogous to PD symptoms in humans. Different strategies, such as measurement of the initiation time of stepping, adjusting step counting, and the vibrissae-elicited forelimb placing tests have been developed in order to evaluate and quantify these symptoms and their relief by drugs (Olsson et al. 1995; Chang et al. 1999; Schallert et al. 2000; Meredith and Kang 2006).

2, 3, and 4 weeks after the unilateral lesioning of the left nigrostriatal pathway with 6-OHDA in rats, the motor performance of the forelimb contralateral to the lesion is significantly and progressively impaired compared with the motor performance of the same forelimb before the lesion. Indeed, hemiparkinsonian rats show marked and long-lasting impairment in the initiation time of stepping movement of the forelimb contralateral to the lesioned side, an impairment considered to be of symptomatic validity for the initiation of movement deficit present in parkinsonian patients (Olsson et al. 1995; Meredith and Kang 2006; Pinna et al. 2007, 2010). Moreover, hemiparkinsonian rats made less steps with the forelimb contralateral to the lesion, compared with their ipsilateral forelimb, showing a marked reduction of movements defined as hypokinesia (Olsson et al. 1995; Chang et al. 1999; Pinna et al. 2007, 2010). Both deficits described were effectively reversed by a dose of L-DOPA at sub-threshold levels for induction of rotation. Similar to L-DOPA, administration of the A_{2A} receptor antagonists SCH58261, SCH420814, ST1535, or ANR94 almost completely counteracted motor initiation deficit and forelimb akinesia/hypokinesia as demonstrated by its effect in improving initiation time of stepping and in increasing the number of steps performed in both a forward and backward direction by the forelimb contralateral to the lesion (Pinna et al. 2007, 2010; Pinna unpublished observations) (Fig. 1; Table 1). However, the improvement with ST1535 was less pronounced than that observed with SCH58261, whereas the effects of ANR94 started with a small delay consistent with the results obtained in haloperidol-induce catalepsy model (Pinna et al. 2007, 2010) (Table 1). Notably, hemiparkinsonian rats did not show any spontaneous recovery in initiation time in the stepping test and in the adjusting test during the period in which the drug-test was performed (Pinna et al. 2007, 2010).

Vibrissae-Placing Test

Similar to parkinsonian patients, hemiparkinsonian rats showed marked sensorimotor integration deficits correlated with unilateral lesion of the dopaminergic nigrostriatal pathway (Schallert et al. 2000). These sensorimotor deficits, assessed by means of the vibrissae-elicited forelimb placing test, hampered the hemiparkinsonian rats when placing their forelimb contralateral to the lesion on the table surface after brushing of the vibrissae on the same side, whereas the ipsilateral forelimb was not affected by the lesion (Schallert et al. 2000; Meredith and Kang 2006; Pinna et al. 2007, 2010). SCH58261, ANR94, and SCH420814, like L-DOPA, completely restored placement of the contralateral forelimb, while ST1535 was effective, but to a lesser extent than the other drugs tested (Pinna et al. 2007, 2010; Pinna unpublished observations) (Fig. 1; Table 1). As described above, hemiparkinsonian rats did not show any spontaneous recovery in sensorimotor integration deficits during the period in which the drug-test was performed.

It is important to underline that even though A_{2A} receptor antagonists do not per se induce contralateral rotations in drug-naïve hemiparkinsonian rats, the behavioral test most utilized for evaluation of antiparkinsonian activity (Fenu et al. 1997; Koga et al. 2000; Morelli and Pinna 2001), they appear, as shown by the results mentioned above, to be effective by themselves in counteracting specific motor deficits associated with dopamine neuron degeneration, such as akinesia/hypokinesia, initiation of movement, and sensorimotor deficits.

Chronic Effects of A_{2A} Antagonists in Hemiparkinsonian Rats

After verification of the antiparkinsonian effectiveness of acutely administered A_{2A} receptor antagonists, it was of fundamental importance to verify their efficacy after chronic treatment, as required by their utilization in a chronic pathology, such as PD. Chronic administration of A_{2A} antagonists has been demonstrated to effectively improve motor deficits in rodent models of PD, and not to produce tolerance to the motor-stimulant effects (Koga et al. 2000; Pinna et al. 2001). In contrast, the non-specific adenosine antagonist caffeine loses its motor-stimulant effect with repeated exposure (Fredholm et al. 1999; Halldner et al. 2000). In hemiparkinsonian rats, a potentiation of the intensity of rotational behavior induced by SCH58261 plus L-DOPA has been observed after either 1 or 2 weeks of repeated daily treatment with this A_{2A} antagonist (Pinna et al. 2001). Furthermore, the potentiation after 2 weeks of SCH58261 treatment was more marked in rats treated with chronic SCH58261 than in those treated with chronic vehicle (Pinna et al. 2001). A similar result has been reported using a combination of KF17837 or KW-6002 with apomorphine, which seems to produce a specific increase in duration rather than in intensity of rotational behavior in hemiparkinsonian rats (Koga et al. 2000). The absence of tolerance to the motor-stimulant effects of A_{2A} antagonists is of greatest interest in a condition requiring a long-term pharmacological treatment, such as PD, in which drugs should retain their motor-facilitating properties over a chronic regimen.

Effects of A_{2A} Antagonists on L-DOPA-Induced Motor Fluctuations and Dyskinesia

Dopamine-replacement therapy, characterized by the dopamine precursor L-DOPA, represents the most widely used and effective treatment for PD. Although L-DOPA is considered the "gold standard" treatment for PD, after several years, neuropsychiatric and motor complications, including fluctuations in motor response and dyskinesias develop in the majority of patients (Olanow 2004). The main limitation of long-term use of L-DOPA in PD treatment is represented by the progressive reduction of the drug's efficacy in counteracting parkinsonian motor symptoms; the conditions commonly observed are "wearing-off" and "ON/OFF" phenomena. During "wearing-off," L-DOPA administration relieves PD motor impairment for a limited period of time, usually a few hours, after which akinesia and rigidity again become evident. In the "ON/OFF" phenomenon, the patient fluctuates from "ON" periods in which the parkinsonian symptoms are counteracted, to "OFF" periods in which the patient shows rigidity and bradykinesia.

Interestingly, findings in hemiparkinsonian rats have shown that, similar to PD patients, the duration of rotational behavior elicited by L-DOPA is progressively reduced during chronic treatment with the drug, a phenomenon that is similar to the L-DOPA "wearing-off" observed in PD patients (Oh and Chase 2002; Marin et al. 2005). As described above, the acute effect of A_{2A} antagonists KF17837, KW-6002, SCH58261, and SCH420814 produced an increased duration of rotational behavior induced by apomorphine or L-DOPA (Koga et al. 2000; Pinna unpublished observations). Consistent with these results, the co-administration of the A_{2A} antagonist KW-6002, SCH420814, or CSC with L-DOPA reversed the shortening of rotational behavior, supporting a potential beneficial influence of A_{2A} blockade on L-DOPA-induced "wearing-off" (Koga et al. 2000; Bibbiani et al. 2003; Bové et al. 2002, 2006; Pinna unpublished observations) (Table 1). However, when CSC was chronically administered in combination with L-DOPA, it did not have any effect in the shortening response of rotational behavior (Bové et al. 2002).

As described above, prolonged use of L-DOPA is associated with the onset of dyskinesias, characterized by abnormal involuntary movements (AIMs), such as chorea and dystonia, which are highly disabling for patients. In hemiparkinsonian rats, sensitization of rotational behavior induced by sub-chronic intermittent treatment of L-DOPA, is a model of dyskinetic effects induced by L-DOPA in humans, since it is only observed after administration on dopamine agonists with high dyskinetic potential (Papa et al. 1994; Henry et al. 1998; Pinna et al. 2001; Carta et al. 2008). In addition, in the same rodent model, the gradual development of AIMs, affecting the forelimb contralateral to the lesion (limb dyskinesia), the trunk (axial dyskinesia), and the orofacial musculature (orolingual dyskinesia), induced by chronic L-DOPA treatment is a well-established model of dyskinesia (Lundblad et al. 2002; Pinna et al. 2006).

In these two paradigms, interesting results concerning the modulation of dyskinesia by A_{2A} receptor blockade have been obtained comparing sensitization of rotational behavior and/or AIMs elicited by long-term treatment of a full dose of L-DOPA with an equipotent combination of a lower dose of L-DOPA plus different A_{2A} antagonists (Pinna et al. 2001; Tronci et al. 2007; Hodgson et al. 2009) (Fig. 1). While both L-DOPA (high dose) and L-DOPA (lower dose) plus SCH58261 or SCH420814 produced a comparable degree of turns on the first administration, sensitization of rotational behavior was observed in response to chronic L-DOPA alone, but not to chronic L-DOPA plus SCH58261 or SCH420814 (Pinna et al. 2001;

Hodgson et al. 2009). Moreover, Lundblad et al. (2003) showed that hemiparkinsonian rats treated with KW-6002 did not develop any AIMs, while relieving motor disabilities assessed by a rotarod test. In addition, in that study, no effect was observed with KW-6002 on the severity of the AIMs induced by repeated L-DOPA at full dose, when the two drugs were chronically co-administered (Lundblad et al. 2003) (Fig. 1). These results suggest that co-treatment with an A_{2A} antagonist plus L-DOPA did not prevent or worsen the occurrence of AIMs when L-DOPA is given at a full dose to severely dopamine-denervated rats (Lundblad et al. 2003). Whereas, chronic co-administration of the A_{2A} antagonist ST1535 with a low dose of L-DOPA did not display any sensitization of both rotational behavior and AIMs (Tronci et al. 2007). The stable response observed after long-term low doses of L-DOPA plus A_{2A} antagonists suggests that the association between the two drugs represents a treatment with a low dyskinetic potential. Interestingly, this hypothesis has been supported by studies showing that genetic deletion of the A_{2A} receptor prevents the sensitization of rotational behavior and AIMs stimulated by L-DOPA in hemiparkinsonian mice (Fredduzzi et al. 2002; Xiao et al. 2006).

Transgenic Models of PD

The findings of gene defects in familial PD have led to the identification of gene products and attempts to produce transgenic models of PD in mice (Duty and Jenner 2011). Although the genetic mice models have not yet contributed to drug discovery for PD, they are nonetheless appreciated models of PD. In the MitoPark mouse, in which dopamine neurons undergo a slow and progressive degeneration due to the cell-type specific induction of mitochondrial dysfunction in midbrain dopaminergic neurons, a chronic treatment with the A_{2A} antagonist MSX-3 prevented the reduction of spontaneous locomotor activity, demonstrating the potential efficacy of A_{2A} antagonists as monotherapy in early PD (Ekstrand et al. 2007; Marcellino et al. 2010).

Conclusions

The results obtained in rodent models of PD suggested that A_{2A} receptor antagonist drugs might have symptomatic therapeutic efficacy in early stages of PD when motor complication are not present yet, since A_{2A} receptor antagonists do not ameliorate dyskinesia. In particular, they suggested that A_{2A} receptor antagonists, when administered alone, may ameliorate initiation of movement, gait and muscle rigidity, at the same time improving the sensorimotor integration deficits and tremor that characterize PD. Moreover, the tests of A_{2A} receptor antagonists in hemiparkinsonian rodents showed that when L-DOPA is administered at a low sub-threshold dose, its efficacy is potentiated. In agreement with this, Bara-Jimenez et al. (2003) reported improvements in motor function with KW-6002, when a low dose of L-DOPA was administered to PD patients, moreover in the same study it was found that tremor was particularly affected.

However, the majority of clinical trials so far performed were in PD patients with L-DOPA-induced complications showing a limited efficacy of this class of drugs with an increase in "ON" periods only, with no exacerbation of dyskinesia (Hauser et al. 2003, 2008, 2011; Stacy et al. 2008; Mizuno et al. 2010). It appears, therefore, that the correspondence between tests performed in rodent models of PD and results in humans, might be limited, raising several questions on how to render the rodent tests more suitable for human application.

On the other hand it would be important to carefully consider the results obtained in rodents before planning a clinical trial. By observing the experience on A_{2A} receptor antagonists in PD, it looks as though lowering of the L-DOPA dosage when concomitantly administering an A_{2A} receptor antagonists was not taken into consideration in the clinical trial planning, as well as the possibility of evaluating the improvement of specific motor deficits or tremor after A_{2A} receptor antagonists.

In addition, animal studies showed that A_{2A} receptor antagonists counteract drug-induced parkinsonism, whereas there is a lack of investigation on this regard in human clinical investigations.

A further concern relates to the use of dietary products, such as caffeine, an antagonist of A_1/A_{2A} receptors, which might interfere with the action of concomitantly administered A_{2A} antagonists. The history of A_{2A} receptor antagonists might, therefore, be taken as an opportunity to reflect on and to refine these actions in order to better link preclinical with clinical studies.

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