Chapter 7
Adenosine A_{2A} Receptor Antagonists as Drugs for Symptomatic Control of Parkinson's Disease in Preclinical Studies

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Abstract Parkinson's disease (PD) is primarily a neurological basal ganglia (BG)-related disorder caused by progressive degeneration of the nigrostriatal dopaminergic neurons, which results in the cardinal motor symptoms of PD, including bradykinesia (slow movement and difficulty in initiation movement), resting tremor, muscle tone rigidity, postural instability, and sensorimotor integration deficits. The gold standard of PD therapy is characterized by the dopamine precursor L-DOPA however, after several years, this therapy leads to neuropsychiatric and motor complications, including fluctuations in motor response and dyskinesias, which develop in the majority of patients. Consequently, one of the main targets of research in PD is to identify alternative therapeutic approaches to ameliorate PD symptoms without inducing motor complications. Among the non-dopaminergic strategies for PD, one of the most promising is represented by adenosine A_{2A} receptor antagonists, due to the colocalization of these receptors and dopamine D_2 receptors in the striatopallidal neurons of the BG, which provides the anatomical basis for the existence of a functional antagonistic interaction between these receptors. Thus, extensive preclinical studies have been performed to prove the effectiveness of adenosine A_{2A} receptor blockade in counteracting the cardinal motor symptoms of PD.

This chapter describes the effects of A_{2A} antagonists alone or in combination with L-DOPA against the cardinal motor symptoms of PD, using rodent and primate models of PD, and the main mechanisms responsible for these anti-parkinsonian effects. In addition, findings suggesting the potential utilization of A_{2A} antagonists, as adjunctive treatments to L-DOPA to reduce the L-DOPA induced *wearing-off* without modifying dyskinetic movements, have been reviewed.

Keywords Parkinson's disease · Rodent models · Non-human primate models · Catalepsy · Rigidity · Tremor · 6-Hydroxydopamine lesion · MPTP lesion · A_{2A} receptor antagonists · Adenosine

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M. Morelli et al. (eds.), *The Adenosinergic System,* Current Topics in Neurotoxicity 10,
DOI 10.1007/978-3-319-20273-0_7

Parkinson's Disease

Parkinson's disease (PD) is the second most common chronic neurodegenerative disease, with a progressive course, affecting over 5 million individuals worldwide (Van Den Eeden et al. 2003). Age is the greatest risk factor for PD, with an average age of onset of approximately 55–65 years (Obeso et al. 2000; Van Den Eeden et al. 2003). The prevalence of PD is expected to rise dramatically over the next 20 years as the population ages (Dorsey et al. 2007).

Symptomatically, PD is characterized by debilitating motor impairment, including akinesia, bradykinesia, muscle rigidity, resting tremor, gait disorders, and postural instability (Marsden 1994; Obeso et al. 2000). Additionally, PD patients are affected by a variety of non-motor symptoms, including cognitive dysfunction, autonomic abnormalities, sleep disturbance, and depression (Chaudhuri et al. 2006).

Pathologically, PD is characterized by degeneration of the nigrostriatal dopaminergic system, which is responsible for many of the motor symptoms observed in the disease. The principal effect of dopaminergic neurodegeneration in the striatum or caudate-putamen (CPu) of parkinsonian patients leads to a disruption of processing in the basal ganglia (BG) circuitry, which is responsible for the integration of sensorimotor information that controls the planning and initiation of voluntary movement (Obeso et al. 2000) (Fig. 7.1). However, neuronal loss has also been observed in brain areas other than the BG, producing changes in neurotransmitters, such as noradrenaline, serotonin, glutamate, acetylcholine, and adenosine, which contribute to the symptomatology of PD (Jellinger 2002). Additionally, widespread Lewy body pathology is observed in both the central and peripheral nervous systems (Braak et al. 2003).

The prime cause of dopaminergic neurodegeneration in PD has not yet been identified, but a large amount of data suggest that, from an aetiological and pathogenetic perspective, it might depend on a combination of environmental and genetic factors, such as toxins, genetic susceptibility, and the aging process (Alves et al. 2008). In particular, several known factors causing PD pathogenesis are mitochondrial dysfunction, oxidative damage, anomalous protein aggregation, and neuroinflammation (Schapira 2006). These processes, once started, persist to cause dopaminergic neuron injury, and have a negative impact on the effectiveness of the current PD therapy.

Since the finding of nigrostriatal dopamine depletion in the BG of parkinsonian patients, the dopaminergic neurotransmitter system has been the main focus of pharmacological therapies for the cardinal features of PD. Dopamine replacement with the dopamine precursor L-DOPA (in combination with a peripheral decarboxylase inhibitor), remains the most efficacious treatment to counteract PD motor symptoms (Olanow et al. 2009). Although L-DOPA is of substantial benefit to antagonize the main motor symptoms in parkinsonian patients, its loses effectiveness over time; specifically, after several years of treatment, the duration of L-DOPA effect shortens (known as *wearing-off*), responses become less predictable (with rapid switching between time spent by patients in a state of mobility *on-time* and immobility *off-time*). Moreover, patients affected by these motor response swings

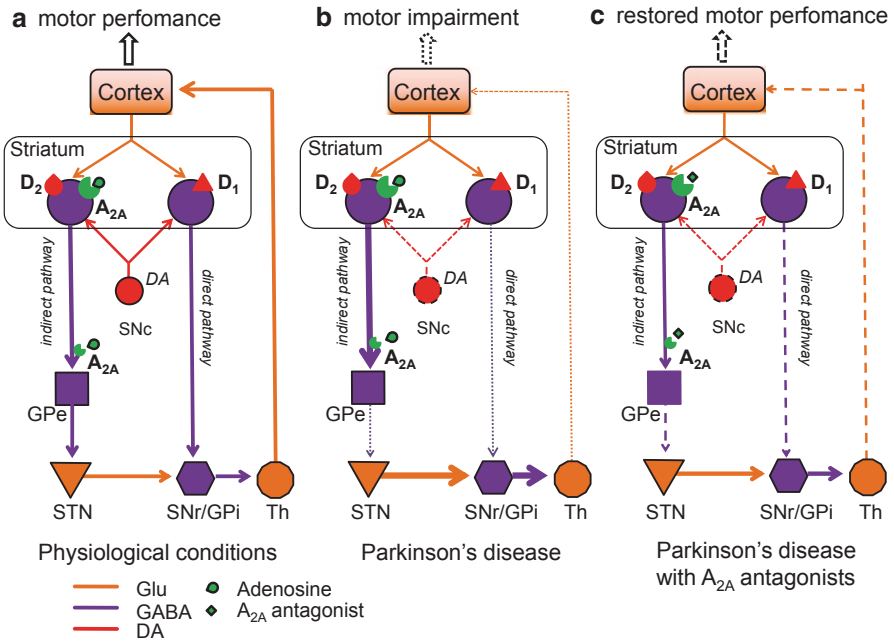

Fig. 7.1 Changes in the function of basal ganglia circuits during Parkinson's disease and effect of adenosine A_{2A} receptor blockade. Under physiological conditions (**a**), the SNc sends dopaminergic inputs to striatal neurons. Endogenous DA then activates the neurons belonging to the striatonigral, or "direct", pathway. These neurons send GABAergic projections to the substantia nigra pars reticulata/globus pallidus pars interna *(SNr/GPi)*, and express stimulatory DA D_1 receptors. At the same time, endogenous DA inhibits the neurons belonging to the striato-pallidal, or "indirect", pathway. These neurons send GABAergic projections to the *SNr/GPi* via globus pallidus pars externa *(GPe)* and subthalamic nucleus *(STN)*, and express inhibitory DA D_2 receptors. The balanced activity of the two striatal efferent pathways underlies the correct execution of movement. Degeneration of SNc neurons during PD removes DA input to the striatum (**b**). This causes the disinhibition of the neurons in the striato-pallidal pathway, and boosts the inhibitory influence these neurons exert on the *GPe*, in turn leading to an overactivation of the *STN* glutamatergic output neurons. The reduction in striatal DA inputs from the SNc also causes a decreased activation of neurons in the striatonigral pathway. Taken together, these modifications in basal ganglia circuits result in an imbalanced activity of the striatal efferent pathways, and in an increased inhibitory output from the *SNr–GPi* complex to the thalamus *(Th)*. As a consequence, the excessive inhibition of thalamocortical neurons causes the motor deficits associated with PD. Adenosine A_{2A} receptors are selectively located in the striato-pallidal pathway, both on striatal medium-sized spiny neurons and on their terminals projecting to GPe (a-b-c). Blockade of these receptors, by counteracting the function of D_2 receptors, alleviates the excessive inhibition of the striato-pallidal pathway, in turn restoring a certain degree of functional balance between the "direct" and "indirect" striatal efferent pathways, and favoring the performance of movement (c)

often show a range of types of choreic or dystonic drug-induced involuntary movements which, in themselves, could become a major source of disability (Olanow et al. 2004) Beside motor fluctuations, neuropsychiatric complications can develop (Obeso et al. 2000; Olanow et al. 2004). Even though, a few pharmacological and surgical strategies exist to ameliorate L-DOPA induced motor complications, they

do not completely solve this problem (Horstink et al. 2006). As a result, the therapy of PD will remain an urgent healthcare issue, and requires an alternative approach to pharmacological intervention that can improve the symptomatology of parkinsonian patients, while, at the same time, offering a lower incidence of adverse effects.

Basal Ganglia Circuitry

The motor BG circuitry involved in the pathophysiology of movement disorders, consist of several subcortical structures, including the striatum or CPu, the globus pallidus (internal [GPi] and external [GPe] divisions), the substantia nigra (pars reticulata [SNr], pars compacta [SNc]), and the subthalamic nucleus (STN) (Delong and Wichmann 2007; Galvan and Wichmann 2008) (Fig. 7.1). All BG-related nuclei are connected through well-established neurochemical circuits, and with the specific cortical areas from which they originate. Briefly, the striatum, under tonic dopaminergic conditions, receives and integrates glutamatergic input from the thalamus and cerebral cortex, and this information is transmitted to the output nuclei, such as the SNr and GPi, which then provide BG projections to the thalamus and cerebral cortex (Delong and Wichmann 2007) (Fig. 7.1). Other BG output nuclei connect with the tegmental pedunculopontine nucleus as well as with the caudal intralaminar nuclei (Delong and Wichmann 2007). The neural population of the striatum is characterized by 95% of medium-sized spiny GABAergic neurons and by 5% of aspiny interneurons, including GABAergic and cholinergic interneurons. The striatal population of medium spiny GABAergic neurons is divided into two neuronal pathways: the monosynaptic "striatonigral direct projection" which connects the striatum with the SNr or GPi and the polysynaptic "striatopallidal indirect projection" which connects the striatum with the GP or GPe (Fig. 7.1). The striatonigral neurons mainly express dopamine D_1 receptors and the neuropeptides substance P and dynorphin, whereas the striatopallidal neurons express predominantly dopamine D_2 receptors and the neuropeptide enkephalin. Dopaminergic input to the striatum arises primarily from the mesencephalon, either from the SNc or the ventral tegmental area, and plays a critical modulatory role in neuronal signalling at this level, exerting a dual effect, depending on the type of post-synaptic dopaminergic receptor stimulated. Specifically, dopamine modulates motor coordination and fine movements by facilitating the direct pathway activity acting on the excitatory dopamine D_1 receptors and by inhibiting the indirect pathway function acting on inhibitory dopamine D_2 receptors (Gerfen and Bolam 2010). In PD, the nigrostriatal dopaminergic neurodegeneration causes dopamine depletion in the striatum, consequently reducing activation of both dopamine D_1 and D_2 receptors (Fig. 7.1). This lack of striatal dopamine generates an imbalance in the activity of striatal output pathways, characterized by reduced excitation of the striatonigral direct pathway, which leads to a decrease in inhibitory control of the GPi/SNr and a concomitant

disinhibition of the striatopallidal indirect pathway to the STN and increases stimulation of the GPi/SNr neurons (Fig. 7.1). Taken together, this sequence of events exacerbates the activation of GABAergic BG output neurons, finally leading to excessive inhibition of thalamocortical projections of the motor systems, causing parkinsonian motor symptoms (Albin et al. 1989; Delong 1990; Obeso et al. 2000) (Fig. 7.1). Although, this proposed model of BG function and dysfunction provides an excellent starting point (Albin et al. 1989; Delong 1990), it is important to highlight that BG organization is far more sophisticated than supposed in this model (reviewed in Bar-Gad and Bergman 2001; Obeso et al. 2000). Thus, recent findings detailing neurotransmission throughout the BG networks should be taken into consideration for a more complete understanding of its organization and activity (Armentero et al. 2011; see also Chap. 2).

Adenosine A_{2A} Receptor Antagonists

The discovery of the restricted expression of adenosine A_{2A} receptors in the BG circuitry and of their close interaction with dopamine, especially with dopamine D_2 receptors, rendered adenosine A_{2A} receptors very attractive as a non-dopaminergic target to be explored for PD therapy. Indeed, adenosine A_{2A} receptors are localized in areas of the BG associated with the dopaminergic nigrostrial and mesolimbic neuronal pathways, including the striatum, GP, nucleus accumbens, and olfactory tubercle (Rosin et al. 1998). Specifically, in the striatum, adenosine A_{2A} receptors are predominantly restricted on the dendritic spines of GABAergic striatopallidal neurons, where they are colocalized with dopamine D_2 receptors (Hettinger et al. 2001), whereas striatonigral neurons do not contain appreciable levels of adenosine A_{2A} receptors (Hettinger et al. 2001) . This colocalization of adenosine A_{2A} and dopamine D_2 receptors in the striatopallidal neurons leads to a functional antagonistic interaction between these receptors (Ferré et al. 1997; Hettinger et al. 2001; Svenningsson et al. 1999). Specifically, stimulation of the dopamine D_2 receptors by dopamine or dopamine D_2 receptor agonists enhances motor activity, whereas stimulation of the adenosine A_{2A} receptors reduces this effect (Ferré et al. 1997). At the biochemical level, this antagonistic functional interaction between adenosine A_{2A} and dopamine D_2 receptors takes place both directly, with an intramembrane receptor–receptor interaction, in which the activation of adenosine A_{2A} receptors decreases the binding affinity of D_2 receptors for dopamine (Ferré et al. 1991) and at the level of second messengers, such as adenylyl cyclase, in which stimulation of adenosine A_{2A} receptors counteracts the dopamine D_2 receptor-mediated inhibition of 3′,5′-cyclic adenosine monophosphate (cAMP) formation and D_2 receptor-induced intracellular Ca^{2+} responses (Hillion et al. 2002; Olah and Stiles 2000; for more details please see also Chaps. 1 and 2).

Modulation of Adenosine A_{2A} Receptors Located in the BG Circuitry

To better understand the anti-parkinsonian efficacy of A_{2A} receptor antagonists on the cardinal symptoms of PD, it is necessary to illustrate the main role played by adenosine A_{2A} receptors in the motor BG circuitry involved in the pathophysiology of movement disorders (Fig. 7.1).

As described above, the colocalization of adenosine A_{2A} and dopamine D_2 receptors in the striatopallidal neurons provides the anatomical basis for the existence of a functional antagonistic interaction between these receptors (Ferré et al. 1997) (Fig. 7.1). Adenosine A_{2A} receptor blockade leads to motor activity by reducing the excessive inhibitory output of the BG indirect pathway, similar to dopamine D_2 receptor activation (Ferré et al. 1997) (Fig. 7.1). In addition, activation or blockade of the adenosine A_{2A} receptors in the indirect striatopallidal pathway, impairs or facilitates dopaminergic D_1-mediated responses as well (Ferré et al. 1997; Pinna et al. 1996; Pollack and Fink 1996). Thus, A_{2A} receptor antagonists seem to restore some balance between the striatonigral and striatopallidal neurons, consequently relieving thalamocortical activity (Fig. 7.1). Moreover, an important function of adenosine A_{2A} receptors has been showed in the GP (Fig. 7.1). Indeed, in PD, the blockade of pallidal adenosine A_{2A} receptors, by reducing extracellular GABA, may contribute to restoring GP activity, and, in turn, STN activity, leading to a more balanced activation of the direct and indirect pathways and, when associated with dopaminergic receptor agonists, an enhancement of their motor-stimulating effects (Ochi et al. 2004; Shindou et al. 2003; Simola et al. 2004, 2008). Furthermore, stimulation of the postsynaptic adenosine A_{2A} receptors antagonizes the inhibitory modulation of the N-methyl-D-aspartate (NMDA) receptor activity mediated by dopamine D_2 receptors (Azdad et al. 2009; Higley and Sabatini 2010). This interaction appears to be responsible for most of the locomotor activation and depression induced by A_{2A} receptor antagonists and agonists, respectively (Ferré et al. 2008). Additionally, further contribution to the anti-parkinsonian effects, in particular the anti-tremorigenic effect, of adenosine A_{2A} receptor antagonists may be related to a cholinergic mechanism (Armentero et al. 2011; Kurokawa et al. 1996; Simola et al. 2004). Indeed, functional antagonism between the adenosine A_{2A} and dopamine D_2 receptors was recently reported in striatal cholinergic interneurons (Tozzi et al. 2011). Moreover, adenosine A_{2A} receptors have been shown to interact either directly or indirectly with various receptors, such as the dopamine D_3, NMDA, cannabinoid CB_1, serotonin 5-HT_{1A}, metabotropic glutamate 4 (mGlu4) and 5 (mGlu5), receptors and to form heteromeric complexes with some of them, suggesting a more complex explanation of their influence on PD motor deficits (Armentero et al. 2011; Bogenpohl et al. 2012; Gerevich et al. 2002; Jones et al. 2012; Łukasiewicz et al. 2007) (see more details in Chap. 2).

The functionally opposing roles of the adenosine A_{2A} and dopamine D_2 receptors on the indirect pathway neurons offers a rationale for the extensive investigation of the activity of A_{2A} receptor antagonists on counteracting motor deficits in pharmacological and toxicological animal models of PD.

The following sections of this chapter illustrate, in detail, the modulatory role played by A_{2A} receptor antagonists on each cardinal PD motor symptom, such as akinesia/bradykinesia, gait impairments, sensorimotor integration deficit, muscle rigidity, and tremor, demonstrated in rodent and primate models of PD.

Effect of A_{2A} Receptor Antagonists on Akinesia, Bradykinesia, and Motor Activity

Akinesia strictly means absence of movement, but in PD, it usually refers to slowness of movement execution (bradykinesia) or lack of spontaneous movements (hypokinesia) (Obeso et al. 2000). In PD, there is a decrease in the amplitude and rate of movements. Bradykinesia may significantly impair the quality of life of PD patients, because it takes much longer to perform everyday tasks, such as eating or dressing. Automatic movements, such as step length or arm swings when walking, or more complex voluntary movements, such as writing or drinking, can all be involved. The effects of A_{2A} receptor antagonists against the symptomatic parkinsonian akinesia, bradykinesia, and motor activity impairment have been demonstrated using a wide range of pharmacological and/or toxicological rodent and primate models of PD, including counteraction of hypomotility or catalepsy induced by haloperidol or reserpine, and modulation of rotational behaviour in rodents, as well as a reduction of motor impairment in non-human primates (Table 7.1 and Fig. 7.2) (Pinna and Morelli 2014; Simola et al. 2008; Xu et al. 2005). In rodents, the monoamine-depleting agent reserpine or the typical neuroleptic haloperidol induces a dramatic reduction of motor activity, principally characterized by akinesia, hypokinesia and catalepsy, which are representative of parkinsonian symptoms and caused by hypofunctionality of the striatum (Duty and Jenner 2011; Gerlach and Riederer 1996). Moreover, rodents administered a range of different doses of reserpine or haloperidol showed other parkinsonian-like symptoms, such as hindlimb rigidity and tremor (as described in the following sections of this chapter) (Duty and Jenner 2011; Gerlach and Riederer 1996; Lorenc-Koci et al. 1996; Salamone et al. 2008). Drugs commonly used in PD treatment are known to counteract the catalepsy induced by haloperidol or reserpine (for review see Duty and Jenner 2011). Moreover, specifically, the catalepsy test induced by haloperidol is useful to underline the pharmacokinetic differences of the compounds tested (Gillespie et al. 2009; Neustadt et al. 2007; Pinna et al. 2005; Weiss et al. 2003).

The majority of A_{2A} receptor antagonists were able to counteract, in a dose-dependent manner, catalepsy and/or hypolocomotion induced by haloperidol or reserpine in rodents, reducing their duration and severity, thereby demonstrating an improvement of parkinsonian motor impairment by these drugs (Table 7.1 and Fig. 7.2) (Drabczyńska et al. 2011; Gillespie et al. 2009; Hodgson et al. 2009; Jones et al. 2013; Kanda et al. 1994; Mandhane et al. 1997; Pinna et al. 2005; Shiozaki et al. 1999; Shook et al. 2010, 2013; Stasi et al. 2006; Villanueva-Toledo 2003; Wardas et al. 2003; Weiss et al. 2003). Furthermore, the co-administration of several

Table 7.1 Summary of the effects exerted by A_{2A} antagonists on cardinal parkinsonian-like symptoms in rodent and primate models of PD

Parkinsonian-like symptoms	Effects of A_{2A} antagonists in rodent models of PD
Parkinsonian-like akinesia, bradykinesia and motor impairment	Reversal of hypolocomotion and catalepsy induced by haloperidol or reserpine (alone or in combination with L-DOPA) [1–14]
	Potentiation of contralateral rotational behaviour induced by DAergic antiparkinsonian drugs in the hemiparkinsonian rats [7, 15–24]
	Restoration of the impaired adjusting steps and initiation time of stepping of the forelimb contralateral to the lesion [19, 25, 26]
	Restoration the lost functionality of hindlimb bradykinesia and in rotarod test of MitoPark mice [27]
Parkinsonian-like sensorimotor integration deficit	Restoration of the placement of the contralateral forelimb after vibrissae brushing lesion [19, 25, 26]
Parkinsonian-like muscle rigidity	Amelioration of parkinsonian-like muscle rigidity produced by either reserpine or haloperidol (alone or in combination with L-DOPA) [14, 28]
Parkinsonian-like tremor	Counteraction of parkinsonian-like tremor elicited by several tremorigenic agents in rodents (for review see Chap. 13 by Salamone)
Parkinsonian-like symptoms	Effects of A_{2A} antagonists in primate models of PD
Parkinsonian-like akinesia, bradykinesia and motor impairment	Reversal of catalepsy induced by haloperidol [29]
	Relieve of motor impairment in MPTP-treated primates (alone or in combination with DAergic antiparkinsonian drugs) [30–34]
Parkinsonian-like muscle rigidity	Amelioration of parkinsonian-like muscle rigidity produced by haloperidol [29]

[1] Drabczyńska et al. 2011; [2] Gillespie et al. 2009; [3] Hodgson et al. 2009; [4] Kanda et al. 1994; [5] Jones et al. 2013; [6] Mandhane et al. 1997; [7] Pinna et al. 2005; [8] Shiozaki et al. 1999; [9] Shook et al. 2010; [10] Shook et al. 2013; [11] Stasi et al. 2006; [12] Villanueva-Toledo 2003; [13] Weiss et al. 2003; [14] Wardas et al. 2003; [15] Fenu et al. 1997; [16] Hodgson et al. 2009; [17] Koga et al. 2000; [18] Pinna et al. 1996; [19] Pinna et al. 2010; [20] Pollack and Fink 1996; [21] Rose et al. 2007; [22] Tronci et al. 2007; [23] Vellucci et al. 1993; [24] Weiss et al. 2003; [25] Pinna et al. 2007; [26] Pinna and Morelli 2014; [27] Smith et al. 2014; [28] Wardas et al. 2001; [29] Varty et al. 2008; [30] Grondin et al. 1999; [31] Hodgson et al. 2010; [32] Kanda et al. 1998; [33] Kanda et al. 2000; [34] Rose et al. 2006

A_{2A} receptor antagonists with L-DOPA has been demonstrated to strengthen the anti-cataleptic effect induced by L-DOPA suggesting that there may be a synergism between the adenosine A_{2A} receptor antagonists and the dopaminergic agents (Table 7.1; Kanda et al. 1994; Shiozaki et al. 1999; Stasi et al. 2006) . Interestingly, Varty and collaborators have also evaluated the efficacy of A_{2A} receptor antagonists against catalepsy induced by haloperidol in primates (Table 7.1 and Fig. 7.2; Varty et al. 2008). The catalepsy induced by haloperidol in primates is characterized by immobility with open eyes, usually accompanied by unusual postures, including rigid limb extensions and/or a twisted torso. Consistent with rodent studies, adenosine A_{2A} receptor blockade can attenuate haloperidol-induced cataleptic motor impairment in monkeys (Table 7.1 and Fig. 7.2; Varty et al. 2008).

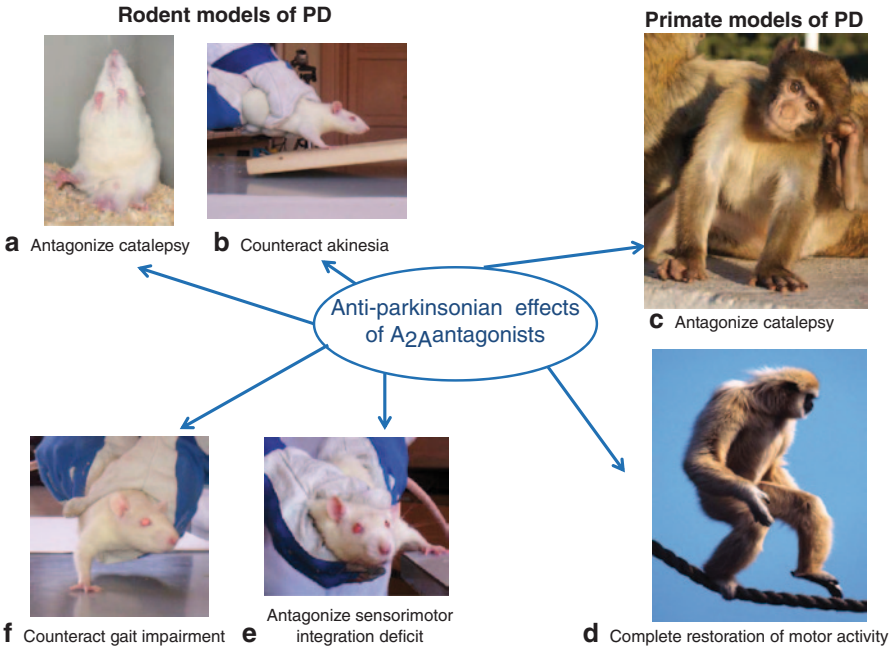

Fig. 7.2 Illustration of diverse rodent and primate models utilized for behavioural evaluation of A_{2A} receptor antagonists. Clockwise shows test performed in rodents *(left panel)* and primates *(right panel)* for **a** catalepsy in rodent, **b** akinesia in rodent, **c** catalepsy in primate, **d** akinesia in primate, **e** sensorimotor integration deficit in rodent, and **f** gait impairment in rodent

To better verify the anti-parkinsonian effects of A_{2A} receptor antagonists, these compounds have been evaluated in the most frequently used PD model of hemiparkinsonian rats, characterized by a unilateral intracerebral infusion of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), which produces massive degeneration of the nigrostriatal dopaminergic neurons, similar to that occurring in idiopathic PD (Schwarting and Huston 1996; Simola et al. 2007; Ungerstedt 1968). In this model, the ability of a specific drug to induce contralateral rotational behaviour, as well as to potentiate the rotational behaviour stimulated by dopamine receptor agonists, can be assumed as a parameter reflecting its anti-parkinsonian activity (Schwarting and Huston 1996; Simola et al. 2007).

A_{2A} receptor antagonists clearly showed a motor facilitative activity in hemiparkinsonian rats. Specifically, acute administration of several adenosine A_{2A} receptor antagonists induced no contralateral rotations *per se*, but significantly potentiated rotational behaviour induced by L-DOPA or apomorphine and by either dopamine D_1 or D_2 receptor agonists, in hemiparkinsonian rodents (Table 7.1; Fenu et al. 1997; Hodgson et al. 2009; Koga et al. 2000; Pinna et al. 1996, 2005, 2010; Pollack and Fink 1996; Rose et al. 2007; Tronci et al. 2007; Vellucci et al. 1993; Weiss et al. 2003).

Furthermore, in hemiparkinsonian rats, more sophisticated measurements of akinesia, bradykinesia, and gait impairment have been assessed. Indeed, as a consequence of unilateral 6-OHDA lesion, rats develop gait impairment and forelimb akinesia considered to be analogous to PD symptoms in humans. Different strategies, such as adjusting step counting and initiation time of stepping have been developed in order to evaluate and quantify these symptoms and their relief by drugs (Chang et al. 1999; Meredith and Kang 2006; Olsson et al. 1995).

A few weeks after unilateral lesioning of the nigrostriatal pathway in 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 made less steps with the forelimb contralateral to the lesion, compared with their ipsilateral forelimb, showing a marked reduction of movements defined as hypokinesia (Chang et al. 1999; Olsson et al. 1995; Pinna et al. 2007, 2010). Moreover, hemiparkinsonian rats show marked and long-lasting impairment in the initiation time of stepping movement of the contralateral to the lesioned side, an impairment considered to be of symptomatic validity for the initiation of movement deficit present in parkinsonian patients (Meredith and Kang 2006; Olsson et al. 1995; Pinna et al. 2007, 2010). Both deficits described were effectively counteracted by a dose of L-DOPA at sub-threshold levels for induction of rotation. Administration of the A_{2A} receptor antagonists, similarly to L-DOPA significantly counteracted forelimb akinesia/hypokinesia and motor initiation deficit, as demonstrated by their effect in increasing the number of steps performed in both a forward and backward direction and in improving initiation time of stepping by the forelimb contralateral to the lesion, with different intensity, depending on the A_{2A} receptor antagonists tested (Table 7.1 and Fig. 7.2; Pinna et al. 2007, 2010; Pinna and Morelli 2014). Notably, hemiparkinsonian rats did not show any spontaneous recovery in the adjusting test and in initiation time in the stepping test during the period in which the drug test was performed (Pinna et al. 2007, 2010).

It is important to underline that even though A_{2A} receptor antagonists do not *per se* induce contralateral rotations in drug-naïve hemiparkinsonian rats, but only potentiate contralateral rotation induced by L-DOPA (Fenu et al. 1997; Koga et al. 2000), they appear, as shown by the above-mentioned results, to be effective in counteracting specific motor deficits associated with dopamine neuron degeneration, such as akinesia/hypokinesia and initiation of movement deficits.

Recently, the anti-akinetic/bradykinetic effects of A_{2A} receptor antagonists have been evaluated in a genetic mouse model of PD that displays a progressive loss of dopamine neurons, such as in the MitoPark mouse (Table 7.1; Smith et al. 2014). The dopamine cell loss in these mice is associated with a deep akinetic phenotype that is sensitive to L-DOPA (Smith et al. 2014). In this PD genetic mouse model, blockade of adenosine A_{2A} receptors increased locomotor activity in a dose-dependent way, completely restored the lost functionality in a measure of hindlimb bradykinesia, and partially restored functionality in a rotarod test, confirming the efficacy of A_{2A} receptor antagonists against these motor deficits (Table 7.1; Smith et al. 2014).

The anti-parkinsonian activity of A_{2A} receptor antagonists against bradykinesia, akinesia, and motor disability shown in the rodent model of PD have been confirmed in a neurotoxic primate model of PD (Table 7.1 and Fig. 7.2).

Primates treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the model of PD, which closely mimics the clinical features of PD in humans, and in which all currently used anti-parkinsonian medications have been shown to be effective; thus, this model is undoubtedly the most clinically relevant of all the available models (Duty and Jenner 2011). Indeed, the MPTP intoxication induced a parkinsonian syndrome, characterized by all of the cardinal symptoms of PD and similar anatomical and functional characteristics of dopaminergic neurodegeneration observed in idiopathic PD (Duty and Jenner 2011). Moreover, the MPTP-treated primate develops clear dyskinesia when repeatedly exposed to L-DOPA and these parkinsonian animals have shown responses to novel dopaminergic agents that are highly predictive of their effect in humans.

Acute administration of A_{2A} receptor antagonists increased locomotor activity and reversed motor disability in a dose-dependent manner in primates previously rendered parkinsonian with MPTP (Table 7.1 and Fig. 7.2; Grondin et al. 1999; Hodgson et al. 2010; Kanda et al. 1998, 2000; Rose et al. 2006). Furthermore, when co-administered with L-DOPA A_{2A} receptor antagonists enhanced the intensity and duration of the efficacy of L-DOPA in reversing motor disabilities and increasing locomotor activity in parkinsonian monkeys (Table 7.1; Hodgson et al. 2010; Kanda et al. 2000; Rose et al. 2006). Similar results have been obtained with the combined administration of A_{2A} receptor antagonists with dopamine D_1 and D_2 receptor agonists (Table 7.1; Kanda et al. 2000). Interestingly, despite producing an enhanced anti-parkinsonian response, acute A_{2A} receptor antagonists did not exacerbate the dyskinesia induced by L-DOPA in MPTP-treated primates previously rendered dyskinetic by L-DOPA exposure (Grondin et al. 1999; Hodgson et al. 2010; Kanda et al. 1998).

Effect of A_{2A} Receptor Antagonists on Sensorimotor Integration Deficit

Similar to parkinsonian patients, hemiparkinsonian rats showed marked sensorimotor integration deficits correlated with a 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 (Meredith and Kang 2006; Pinna et al. 2007, 2010; Schallert et al. 2000). A few A_{2A} receptor antagonists, similarly to L-DOPA completely restored placement of the contralateral forelimb by rats, with different intensity depending on the different A_{2A} receptor antagonists tested, suggesting a potential efficacy of these compounds to ameliorate the sensorimotor integration deficits in PD

patients (Table 7.1 and Fig. 7.2; Pinna and Morelli 2014; Pinna et al. 2007, 2010). This effect was not due to spontaneous recovery of sensorimotor integration deficits by hemiparkinsonian rats (Pinna and Morelli 2014; Pinna et al. 2007, 2010).

Effect of A_{2A} Receptor Antagonist on Muscle Rigidity

The other cardinal symptom of PD, as frequently disabling for patients as bradykinesia and akinesia, is muscle rigidity, which is clinically defined as a sustained increase in resistance to passive movement of a joint throughout its range (Delwaide 2001). The most common clinical characteristic of rigidity is an increased resistance to passive movement of the PD patient's limbs, usually associated with a cogwheel phenomenon, and which could be reproduced in rodents by administration of adequate doses of haloperidol, reserpine, or bilateral 6-OHDA into the SN (Lorenc-Koci et al. 1995, 1996). Both haloperidol and reserpine evoke a muscle rigidity with a lot of electromyographic (EMG) and mechanographic (MMG, muscle resistance) peculiarities similar to those observed in PD patients (Lorenc-Koci et al. 1995, 1996; Wolfarth et al. 1996). Specifically, such rigidity develops in response to passive movements and is characterized by increased resistance of rodent hindlimbs to passive displacement, potentiation of EMG components, and co-activation of antagonistic muscles in response to passive movements. Moreover, as in parkinsonian patients, a tonic EMG activity develops at rest, which reflects some difficulty in relaxing the muscles (Lee 1989).

This combined EMG and MMG method to measure haloperidol or reserpine-induced muscular rigidity has been validated by the fact that muscle rigidity can be reduced by anti-parkinsonian dopaminomimetic agents, including L-DOPA (Wardas et al. 2001).

Although a generic effect of A_{2A} receptor antagonists on counteracting postural rigidity, one of the components of catalepsy induced by haloperidol or reserpine, has been shown in the above section of this chapter, a more precise evaluation of the anti-parkinsonian-like muscular rigidity exerted by these compounds has been made by means of this combined EMG and MMG method (Table 7.1; Wardas 2003; Wardas et al. 2001).

Blockade of adenosine A_{2A} receptors counteracted both components (EMG and MMG) of muscle rigidity induced by haloperidol or reserpine plus alpha-methyl-p-tyrosine in rodents (Table 7.1; Wardas 2003; Wardas et al. 2001). Furthermore, the blockade of adenosine A_{2A} receptors potentiated the alleviating effect of a low dose of L-DOPA which alone did not affect the reserpine- or haloperidol-induced muscular rigidity (Table 7.1; Wardas 2003; Wardas et al. 2001). These beneficial effects on parkinsonian-like muscular rigidity of A_{2A} receptor antagonists have been suggested to be mediated by the facilitation of dopamine transmission at the postsynaptic level, as described above (Wardas 2003; Wardas et al. 2001). Moreover, although, the study by Varty et al. (2008) did not perform a specific measure of haloperidol-induced muscle rigidity by means of suitable equipment, counteraction

of this symptom was observed in primates after combined administration of A_{2A} receptor antagonists (Table 7.1 and Fig. 7.2; Varty et al. 2008). These findings regarding the effectiveness of A_{2A} receptor antagonists on muscle rigidity in rodent and primate PD models indicate that these drugs might be particularly effective in counteracting parkinsonian-like muscle rigidity in PD patients, which is often resistant to common anti-parkinsonian drugs.

Effect of A_{2A} Receptor Antagonist on Tremor Model of PD

Another important anti-parkinsonian effect exerted by A_{2A} receptor antagonists is the anti-tremorigenic effect (Table 7.1). Indeed, tremor is one of the cardinal symptoms of parkinsonism, which is experienced by more than 70% of PD patients (Deuschl et al. 2012). Tremor in PD is typically resting and disappears when voluntary movement is performed. The distal joints of the limbs are preferentially affected. Tremor can be intermittent and is increased by stress (Deuschl et al. 2012). In addition, resting tremor has been shown to respond poorly to traditional anti-parkinsonian medications, including L-DOPA (Jiménez and Vingerhoets 2012). Therefore, research addresses improving the management of this disturbance. To date, experimental models of parkinsonian tremor characterized by tremulous jaw movements (TJMs) induced by several drugs, such as acetylcholinesterase inhibitors, muscarinic agonists, DA receptor antagonists, and neurotoxic degeneration of DA neurons, have been validated for evaluating the anti-tremorigenic effects of drugs (Cousins et al. 1997; Ishiwari et al. 2005; Salamone et al. 1998; Simola et al. 2004; for review see Chap. 8). These tremorigenic compounds produced TJMs which possess many of the pharmacological and electromyographic characteristics of the parkinsonian tremor in humans (for review see Collins-Praino et al. 2011; Chap. 8). Acute administration of several A_{2A} receptor antagonists significantly reversed jaw tremor induced by tacrine, pilocarpine, haloperidol, reserpine, and pimozide in rats, suggesting a beneficial use of these compounds as specific drugs against this parkinsonian symptom (Table 7.1; Betz et al. 2009; Collins et al. 2010, 2012; Collins-Praino et al. 2011; Correa et al. 2004; Pinna et al. 2010; Salamone et al. 2008; Simola et al. 2004, 2006; Tronci et al. 2007). Consistent with these findings, A_{2A} receptor antagonism or genetic deletion of the adenosine A_{2A} receptor significantly attenuated the TJMs induced by pilocarpine in mice (Table 7.1; Salamone et al. 2013). The anti-tremorigenic effect of A_{2A} receptor antagonists appears to be focused particularly on the ventrolateral portion of the striatum, in which a specific increase in adenosine A_{2A} receptor mRNA expression was detected following dopamine denervation in hemiparkinsonian rats (Pinna et al. 2002; Simola et al. 2004). Considering the important role played by an increase in striatal acetylcholine in tremor development, and the reduction of the evoked release of this neurotransmitter exerted by A_{2A} receptor antagonists, it might be suggested that modulation of this anticholinergic effect by blockade of the adenosine A_{2A} receptors may explain its anti-tremorigenic effect (Simola et al. 2004, 2006). Recently, it has been

suggested that A_{2A} receptor antagonists might also be used to modulate the anti-tremorigenic effect of STN deep brain stimulation in PD patients (for details see Chap. 8) (Collins-Praino et al. 2013). Taken together, these data concerning the efficacy of A_{2A} receptor antagonists achieved in rodent models of parkinsonian tremor show that A_{2A} receptor antagonism might be useful to attenuate parkinsonian-like resting tremor, a symptom hardly managed (for details see Chap. 8).

Persistent Efficacy of A_{2A} Receptor Antagonists on Cardinal Symptoms of PD

Specific studies have thus been performed to verify whether the symptomatic anti-parkinsonian acute effects of A_{2A} receptor antagonists persist over time during repeated treatment in animal models of PD, as required by their utilization in a chronic pathology, such as PD. Indeed, discouraging results have been provided by the non-specific adenosine receptor antagonist caffeine, which loses its motor-stimulant effect during chronic exposure (Fredholm et al. 1999; Halldner et al. 2000). In contrast to caffeine, chronic administration of A_{2A} receptor antagonists has been demonstrated to effectively improve motor deficits in rodent and primate models of PD, and not to produce tolerance to their motor-stimulant effects (Kanda et al. 1998; Koga et al. 2000; Pinna et al. 2001). Interestingly, repeated treatment with A_{2A} receptor antagonists for 1 and 2 weeks produced not merely tolerance, but also led to an enhancement of the intensity of the L-DOPA induced rotational behaviour compared with that observed after acute administration of A_{2A} receptor antagonists in hemiparkinsonian rats (Pinna et al. 2001). Similarly, combined administration of A_{2A} receptor antagonists with apomorphine produced a specific increase in duration rather than in intensity of rotational behaviour in hemiparkinsonian rats (Koga et al. 2000). Moreover, the long-lasting efficacy of A_{2A} receptor antagonists in preventing the reduction of spontaneous locomotor activity has recently been demonstrated in both early (8 weeks of age) and mild to severe (12–22 weeks of age) parkinsonian genetic MitoPark mice (Marcellino et al. 2010; Smith et al. 2014).

Effects of A_{2A} Receptor Antagonists on L-DOPA Induced Motor Fluctuations Like Wearing-off and on–off Phenomena

The main limitation of long-term therapy with L-DOPA in PD patients is characterized by motor fluctuations consistent with the progressive reduction of the drug's efficacy in preventing parkinsonian motor symptoms, usually known as "*wearing-off*" and "*on–off*" phenomena (Olanow et al. 2004). During *wearing-off*, L-DOPA counteracts PD motor deficits for a shorter period of time, after which akinesia and rigidity become manifest again. In the *on–off* phenomenon, the patient fluctuates

from "*on*" periods in which the parkinsonian impairments are counteracted, to "*off*" periods in which the patient shows bradykinesia and rigidity. In hemiparkinsonian rats, the duration of rotational behaviour induced by L-DOPA progressively decreases during the long-term treatment with this drug, a phenomenon that mimics the *wearing-off* of L-DOPA observed in parkinsonian patients (Marin et al. 2005; Oh and Chase 2002). Consistent with the acute effect of A_{2A} receptor antagonists producing an increased duration of rotational behaviour induced by L-DOPA or apomorphine (Koga et al. 2000; Pinna and Morelli 2014), the co-administration of the A_{2A} receptor antagonists with L-DOPA reversed the shortening of rotational behaviour, supporting a potential beneficial influence of adenosine A_{2A} receptor blockade on L-DOPA induced *wearing-off* (Bibbiani et al. 2003; Bové et al. 2002; 2006; Koga et al. 2000; Pinna and Morelli 2014). However, when the A_{2A} receptor antagonist 8-(3-chlorostyryl)caffeine was chronically administered in combination with L-DOPA it seems to reverse, but not to prevent, the shortening response of rotational behaviour induced by repeated treatment with L-DOPA (Bové et al. 2002) . Despite this controversial single study, numerous clinical trials in advanced PD patients have demonstrated the efficacy of A_{2A} receptor antagonists in reducing the *wearing off* phenomenon and in increasing the *on period* (for review see Chap. 14), providing effort to approve the commercialization of the A_{2A} receptor antagonist istradefylline as a drug to counteract *wearing-off* in PD patients (for details see Chap. 13).

Effects of A_{2A} Receptor Antagonists on L-DOPA Induced Dyskinesia

Chronic therapy with L-DOPA is associated with the development of dyskinesia, characterized by abnormal involuntary movements (AIMs), such as dystonia (a painful, involuntary spasm of muscles in various parts of the body) and chorea (brief semi-directed, irregular movements that are not repetitive or rhythmic, but appear to flow from one muscle to the next), which are highly disabling for parkinsonian patients (Olanow et al. 2004). As described extensively in the Chap. 9 by Morelli, the influence of adenosine A_{2A} receptor blockade on dyskinesia has been investigated by means of validated experimental paradigms in which dyskinetic movements induced by chronic L-DOPA are expressed both in hemiparkinsonian rodents (sensitization of rotational behaviour and/or AIMs affecting parts of the body contralateral to the lesion) (Henry et al. 1998; Lundblad et al. 2003; Pinna et al. 2001; Tronci et al. 2007), and in parkinsonian MPTP-treated primates (dyskinetic movements affecting several parts of the body, similar to those observed in parkinsonian patients) (Bibbiani et al. 2005). Summarizing the main findings concerning dyskinesia, it has been demonstrated that A_{2A} receptor antagonists, when administered alone, did not induce dyskinesia in both rodents and primates previously rendered dyskinetic by chronic L-DOPA (Grondin et al. 1999; Hodgson et al. 2010; Jones et al. 2013; Kanda et al. 1998; Lundblad et al. 2002). Moreover, in

hemiparkinsonian rats, long-term treatment with a combination of an A_{2A} receptor antagonist and low doses of L-DOPA induced a stable response in both rotational behaviour and AIMs, suggesting that this association between the two drugs represents a treatment with a low dyskinetic potential (Hodgson et al. 2009; Pinna et al. 2001; Tronci et al. 2007). Conversely, blockade of the adenosine A_{2A} receptors did not produce any effect on the severity of the AIMs induced by repeated L-DOPA at full dose, when the two drugs were chronically co-administered in hemiparkinsonian rats (Jones et al. 2013; Lundblad et al. 2003). Interestingly, this hypothesis has been supported by studies showing that genetic deletion of the adenosine A_{2A} receptor prevents the sensitization of rotational behaviour and AIMs stimulated by L-DOPA in hemiparkinsonian mice (Fredduzzi et al. 2002; Xiao et al. 2006). Findings in dyskinetic parkinsonian primates confirmed that A_{2A} receptor antagonists associated with a low non-dyskinetic dose of L-DOPA may ameliorate satisfactory motor deficits, limiting the severity of L-DOPA induced dyskinesia (Hodgson et al. 2010; Kanda et al. 2000). Taken together, these results suggested that although no study has yet demonstrated the ability of A_{2A} receptor antagonists to revert an already established dyskinesia in both rodents and primates, the association of A_{2A} receptor antagonists with a low non-dyskinetic dose of L-DOPA might produce an efficient improvement of motor symptoms, with a concomitant reduction of the intensity of dyskinetic movements (for details see Chap. 9).

Conclusions

In conclusion, data reported in the present chapter describe A_{2A} receptor antagonists as being extremely promising compounds to be used in the therapy of PD. Their potential is largely represented by the marked efficacy demonstrated in alleviating every cardinal PD motor symptom observed in pharmacological and toxicological animal models of PD. The findings achieved in both rodent and primate models of PD suggested that A_{2A} receptor antagonist agents might have symptomatic therapeutic effectiveness in the early stages of PD, when motor complications have not yet appeared, because A_{2A} receptor antagonists do not counteract dyskinesia. Specifically, they suggested that A_{2A} receptor antagonists, *per se*, may improve akinesia/bradykinesia, initiation of movement and gait impairments, and muscle rigidity, and, at the same time, ameliorate the sensorimotor integration deficits and tremor that characterize PD. Moreover, experiments performed in hemiparkinsonian rodents demonstrated that the combined administration of A_{2A} receptor antagonists with a low sub-threshold dose of L-DOPA potentiated the effect of L-DOPA Moreover, the persistent anti-parkinsonian efficacy of A_{2A} receptor antagonists during chronic treatment is of greatest interest in a condition requiring long-term pharmacological management, such as PD, in which drugs should retain their motor-facilitating properties over a chronic regimen. In addition, experimental data show the efficacy of A_{2A} receptor antagonists in reducing the *wearing off* phenomenon and in increasing the "*on periods"* with no exacerbation of dyskinesia.

Altogether, these preclinical studies demonstrate the need to investigate, through clinical trials, the potential utilization of A_{2A} receptor antagonists in the management of the cardinal symptoms of parkinsonian patients, both as monotherapies and in combination with low doses of L-DOPA.

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