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9 Adenosine A_{2A} receptor antagonist treatment of Parkinson's disease

Annalisa Pinna[1,2§], Nicola Simola[2§] and Micaela Morelli[1,2]
[1]CNR Institute for Neuroscience – section of Cagliari, Italy; [2]Department of Toxicology and Centre of Excellence for Neurobiology of Dependence University of Cagliari, 09124 Cagliari, Italy

Abstract

Adenosine A_{2A} receptors have a unique cellular and regional distribution in the basal ganglia (BG), being particularly concentrated in areas richly innervated by dopamine (DA) such as the caudate-putamen, otherwise called striatum, and the globus pallidus. Adenosine A_{2A} and DA D_2 receptors are capable of forming functional heteromeric complexes and are colocalised in striatopallidal neurons. Based on the peculiar cellular and regional distribution of this receptor and in line with data showing that A_{2A} receptor antagonists

[§]both authors contributed equally

Correspondence/Reprint request: Prof. Micaela Morelli, Department of Toxicology and Centre of Excellence for NeuroBiology of Dependence, University of Cagliari, Via Ospedale, 72, 09124 Cagliari, Italy
E-mail: morelli@unica.it

improve motor symptoms of Parkinson's disease (PD) in animal models and in clinical trials, A_{2A} receptor antagonists have emerged as an attractive non-dopaminergic target to improve the motor deficits that characterise PD. Experimental data have also shown that A_{2A} receptor antagonists are capable of exerting a neuroprotective effect and do not induce neuroplasticity phenomena that complicate long-term dopaminergic treatments. The present review will provide an updated summary of results reported in the literature concerning the biochemical characteristics and BG distribution of A_{2A} receptors. We subsequently aim to examine the effects of adenosine A_{2A} antagonists in rodent and primate models of PD and L-DOPA-induced dyskinesia. Finally, conclusive remarks will be made on the neuroprotective effects of A_{2A} antagonists and on the translation of adenosine A_{2A} receptor antagonists in the treatment of PD.

Introduction

Adenosine is an endogenous nucleoside, made up of the purine base adenine linked to ribose, present in the intra and extracellular spaces of all mammalian tissues where it takes part in a large number of physiological processes.

Adenosine formation critically depends on ATP breakdown and synthesis. At intracellular level adenosine is formed through hydrolysis of AMP by means of the enzyme 5'-nucleotidase, or alternatively in lower concentrations through hydrolysis of S-adenosylhomocysteine (SAH) by a specific hydrolase. Conversely, extracellular adenosine levels appear to be dependent on both intracellular adenosine outflow and on the degradation of extracellularly released nucleotides. The balance of adenosine concentrations in the intra and extracellular spaces is maintained by means of specialized bi-directional nucleoside transporters. Inactivation of extracellular adenosine is primarily mediated by uptake across the neuronal cell membrane, followed by either phosphorylation into AMP by adenosine kinase or deamination into inosine by adenosine deaminase. Another possible catabolic pathway is represented by a reversible reaction catalysed by SAH hydrolase, forming SAH from adenosine and L-homocysteine [1].

In the Central Nervous System (CNS), adenosine is released by neurons and glial cells as a result of metabolic activity [1]. The normal extracellular levels of adenosine in the CNS are in the medium nanomolar range (30-300 nm), although this concentration may rise as much as one hundred-fold as a result of oxygen limitation, such as occurs during hypoxia or ischemia [2]. Adenosine exerts a dual modulatory role at either pre or postsynaptic level acting as a homeostatic modulator by controlling neurotransmitter release and neuronal responses [3].

The adenosine receptors

The effects of adenosine are mediated through specific receptors belonging to the family of G protein-coupled receptors (GPCR) located on cell membranes and

characterized by the presence of seven transmembrane domains. To date, four different subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) have been cloned and characterized in several species including humans [4]. A_3 and A_{2B} receptors, which display a low affinity for adenosine, are thought to be stimulated only by the high concentrations of adenosine reached during pathological conditions (micromolar range) [4,5]. On the other hand, the A_1 and A_{2A} receptor subtypes bind adenosine with high affinity, their stimulation being involved in the physiological action of the neuromodulator [4]. Accordingly, A_1 and A_{2A} subtypes are the most widely studied among the adenosine receptors.

The main intracellular signalling pathways utilized by adenosine receptors involve the modification of cAMP levels, A_1 and A_3 receptors causing inhibition of adenylate cyclase and A_{2A} and A_{2B} receptors activating the enzyme [4]. Other mechanisms such as regulation of voltage-sensitive Ca^{2+} channels or G protein-dependent K^+ channels, also participate in signal transduction by each of the adenosine receptors [4].

Distribution of A_{2A} receptors in the brain

Several autoradiographic studies performed using radiolabelled ligands, *in situ* hybridization and reverse transcription polymerase chain reaction have shown that A_1 and A_{2A} receptors are expressed at high levels in the brain, although with different distributions. Whereas A_1 receptors are widely expressed throughout the brain, A_{2A} receptors are highly enriched in the basal ganglia (BG) nuclei where they play an important role in the control of motor behavior, a feature of particular relevance in Parkinson's disease (PD) (Fig. 1).

Autoradiographic studies using different radioligands have shown high levels of A_{2A} receptor in the caudate-putamen (CPu) or striatum, nucleus accumbens (NAc), olfactory tubercles, and the external globus pallidus (GPe) in rat and human brain [6-9]. Consistently, immunohistochemical studies, using both monoclonal antibody generated against purified recombinant human A_{2A} receptor [10] and polyclonal antibody against rat A_{2A} receptor [11] substantially confirmed this selective localization of the A_{2A} receptor in rat brain. These findings have been substantiated by results deriving from *in situ* hybridization studies showing that in rodent, primate and human the CPu, NAc, and the olfactory tubercles are enriched in mRNA encoding A_{2A} receptors [12-17].

Moreover, using more sensitive techniques, lower levels of A_{2A} receptors and their relative mRNAs have also been shown in several other brain areas outside the BG such as the hippocampus, the cerebral cortex, the thalamus and the cerebellum, with differences being observed between humans and other animal species [8-11,15,16]. Recently, the presence of A_{2A} receptors has been described in detail in astrocytes in various brain areas [11,18]. It should be pointed out how, in spite of the use of different methodological approaches, all the above mentioned studies are consistent in describing high levels of A_{2A} receptors in the striatum, an area extensively involved in the control of movement [19] (Fig. 1).

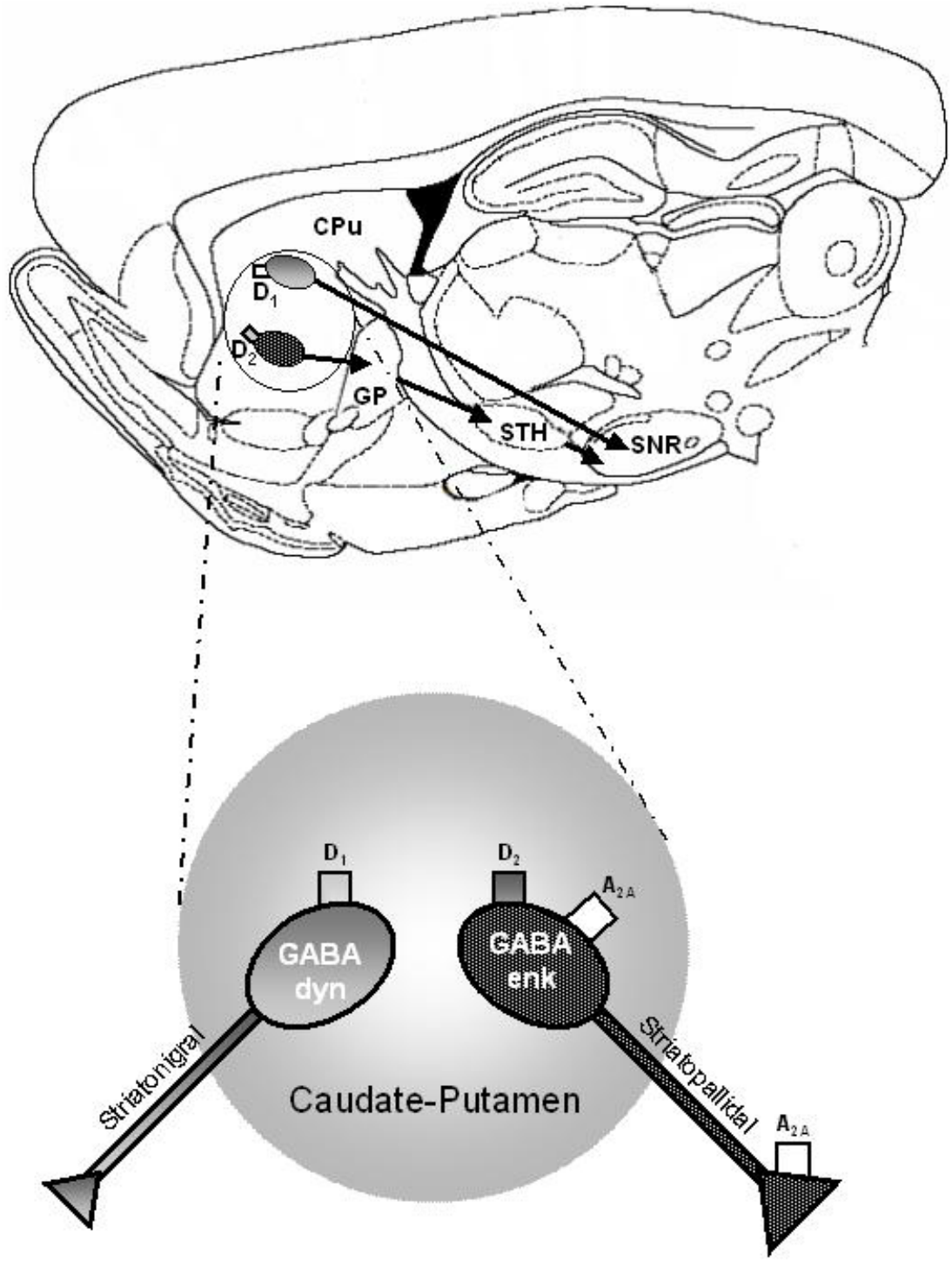


Figure 1. Schematic representation of regional (sagittal section of rat brain) and cellular localization of A_{2A} receptor in the Caudate-Putamen (CPu) that provide the basis of antiparkinsonian action of A_{2A} antagonists. As showed in this figure representing the two major CPu GABAergic output pathways A_{2A} receptors are largely restricted to GABAergic neurons that express dopamine D_2 receptors and enkephalin (enk) and project to the globus pallidus (GP). By contrast, GABAergic neurons projecting directly to substantia nigra reticulata (SNR) are enriched in D_1 receptors and dynorphin (dyn) and do not appreciably express A_{2A} receptors.

Basal ganglia organisation and neuronal localisation of A_{2A} receptors

To better understand the means by which adenosine A_{2A} receptors influence motor functions, their role in the neuronal circuitry of BG, a group of brain nuclei implicated in motor function and in pathophysiology of movement disorders such as PD, should be clarified. As mentioned previously, A_{2A} receptors are present in many BG nuclei, likely affecting motor behaviour by acting at different levels of the BG network.

The BG comprise the CPu, the GPe, internal segment of the globus pallidus (GPi), substantia nigra pars compacta (SNc) and reticulata (SNr) and the subthalamic nucleus (STN). The major neural population in the CPu is represented by medium-sized spiny projection neurons (MSNs), accounting for almost 95% of striatal neurons and using γ-amino-butyric acid (GABA) as neurotransmitter. The remaining 5% of striatal cells consists of aspiny interneurons, including GABAergic and cholinergic interneurons. The activity of MSNs is regulated by two main inputs: dopaminergic projection from the SNc and glutamatergic input from cortical, thalamic and limbic areas [20]. Moreover, the GABAergic spiny neurons give rise to the two main striatal efferent circuits: the striatonigral and the striatopallidal pathway. The neurons of the striatonigral, or direct, pathway contain the neuropeptides substance P and dynorphin (DYN) and mainly express D_1 receptors; this pathway directly projects from the CPu to the GPi/SNr (Fig. 1).

On the other hand, the neurons of the striatopallidal, or indirect, pathway containing the neuropeptide enkephalin (ENK), predominantly express D_2 receptors; this circuit connects the striatum with the GPi/SNr via synaptic connections in the GPe and STN [20] (Fig. 1). DA modulates motor coordination and fine movements by facilitating the action of the direct pathway on stimulatory D_1 receptors and by inhibiting indirect pathway function acting on inhibitory D_2 receptors [20].

In the rat BG, A_{2A} receptors are homogeneously distributed throughout the lateral (motor portion), medial (associative portion) and ventro-medial (limbic portion) CPu. An important peculiarity of adenosine A_{2A} receptors is their expression almost exclusively on striatopallidal neurons, where they are colocalized and interact strictly with D_2 receptors [14,17,21] (Fig. 1). In contrast, a very few striatonigral neurons expressing D_1 receptors were found to co-express A_{2A} receptors [16,17,21,22]. A_{2A} receptor mRNA was also detected in striatal cholinergic interneurons [23,24]. A_{2A} receptors are almost exclusively restricted to the dendritic spines of striatopallidal neurons, especially in the vicinity of the glutamatergic synapse. Moreover, A_{2A} receptors are found in lower concentrations presynaptically on the glutamatergic terminals, postsynaptically around the dopaminergic synapse

and on glial sites [18]. In contrast, in the substantia nigra (SN) A_{2A} receptors are mostly concentrated in the perikarya of the SNc and SN pars lateralis neurons. The STN is the BG nucleus having the lowest concentration of A_{2A} receptors, their distribution being restricted to the perikarya [10].

Interaction between A_{2A} and dopamine receptors

In the BG a wide interaction between DA and A_{2A} receptors has been described, suggesting that these latter receptors extensively modulate BG functionality.

The colocalization of adenosine A_{2A} and D_2 receptors in the striatopallidal neurons provides an anatomical basis for the existence of a functional antagonistic interaction between these receptors (Fig. 1). Results from several studies have shown that adenosine A_{2A} receptors exert an excitatory influence on striatopallidal neurons, which is partially related to their antagonistic effect on D_2 receptor activation [25].

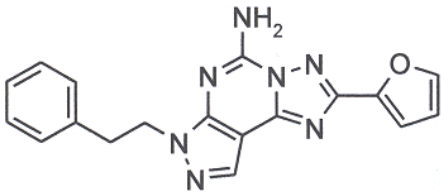
The first biochemical evidence of an A_{2A}/D_2 interaction was shown in rat striatal membrane where the activation of adenosine A_{2A} receptors decreased the binding affinity of D_2 receptors for DA [26]. Later, the same effect was also demonstrated in human striatal tissue [27,28] and in different cell lines [29-31]. These effects are likely due to the formation of heterodimeric complexes between the two receptors [32]. Moreover, the A_{2A}/D_2 interaction seems to be more potent in the DA-denervated striatum with supersensitive D_2 receptors [33]. Consistently, stimulation of adenosine A_{2A} receptors counteracts the D_2 receptor-mediated inhibition of cAMP formation and D_2 receptor-induced intracellular Ca^{2+} responses [30,31,34].

Despite the fact that A_{2A} receptors generally couple to Gs proteins in peripheral tissues, Kull et al. [35] have demonstrated a coexpression of A_{2A} receptors with Golf proteins in GABAergic striatopallidal neurons, moreover demonstrating how stimulation of A_{2A} receptors activates Golf in rat striatal membrane. These findings indicate that A_{2A} receptors may be coupled to specific G proteins in different areas. Thus, stimulation of D_2 receptors inhibits adenylyl cyclase through Gi proteins, whereas stimulation of A_{2A} receptor, coupled to Gs/olf proteins, activates adenylyl cyclase. This strong antagonistic A_{2A}/D_2 receptor interaction at the adenylyl cyclase level leads to an opposite regulation of the activity of cAMP-dependent protein kinase (PKA) which, in turn, controls the phosphorylation state and activity of various receptors, ion channels, phosphodiesterases and numerous phosphoproteins. Consequently, the regulatory activity exerted by A_{2A} receptors on the DA and cAMP-regulated phosphoprotein (DARPP-32), which is deeply involved in DA-mediated signal is of particular interest. As well as the activity it exerts on transcription factors, such as the cAMP-responsive element binding protein (CREB), which controls the expression of immediate early genes.

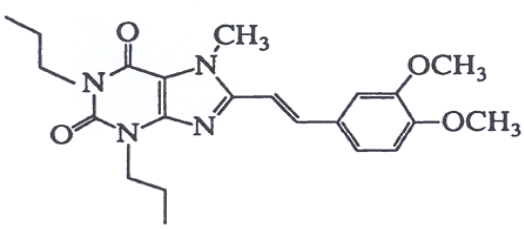
The phosphoprotein DARPP-32 is expressed in very high concentrations in the GABAergic efferent neurons and can be phosphorylated into two different threonin (Thr) residues, namely Thr-34 and Thr-75, resulting in an amplification or inhibition of the PKA signal transduction, respectively [36-38]. Stimulation of A_{2A} receptors produces, in rat striatal slices, phosphorylation of DARPP-32 at Thr-34 and dephosphorylation of DARPP-32 at Thr-75 through cAMP accumulation and PKA stimulation [39-42]. Consistently, Thr-34 phosphorylation of DARPP-32 induced by A_{2A} receptor stimulation was found to be completely counteracted by D_2 receptor agonists [41]. Moreover, studies in mice bearing a genetic deletion of DARPP-32 confirmed the involvement of this phosphoprotein in A_{2A} receptor signalling, revealing an attenuation of the motor-stimulant effects induced by the non selective adenosine receptor antagonist caffeine as well as by the selective A_{2A} antagonist SCH 58261 [42] (Fig. 2).

Another highly relevant issue in the regulation of signal transduction by A_{2A} receptors is represented by the regulation of CREB activity. Thus, A_{2A}-mediated induction of cAMP releases the catalytic subunits of PKA, which diffuse into the nucleus and induce cellular gene expression by phosphorylating CREB at a specific serine residue (Ser-133) [43]. In Chinese hamster ovary cells cotransfected with the A_{2A} and D_2 receptor cDNAs, stimulation of A_{2A} receptors produced a high increase in cAMP formation and phosphorylation of CREB, followed by an induction of *c-fos* mRNA [29]. Quinpirole, a selective D_2/D_3 agonist, dose-dependently counteracted these effects, achieving a complete blockade, at low concentrations, of the D_2 agonist [29]. Accordingly, a variety of *in vivo* studies supports the reciprocal antagonistic influence of A_{2A} and D_2 receptor in the induction of immediate early-gene expression (e.g., *c-fos*, *zif/268*, NGFI-B and *jun-B*) [34, 44-49]. Despite the above findings, it has however been shown that stimulation, as well as blockade, of adenosine A_{2A} receptors induces behavioral and biochemical responses in mice lacking D_2 receptors, thus suggesting that adenosine A_{2A} receptor actions can occur, at least in part, independently from DA [50-52].

In-depth knowledge of the biology of the A_{2A} receptor with specific concern for its molecular interactions with receptors for different neurotransmitters was reached with the discovery of functional heteromeric receptor complexes (receptor mosaics) formed by the A_{2A} receptor with other GPCRs, such as D_2 and glutamate mGlu5 receptors. This finding constituted a considerable step forward in adenosine neurobiology and suggested new ways of influencing the regulation of neuronal activity by A_{2A} receptor manipulation [53]. A number of new approaches have provided convincing evidence for the existence of heteromeric receptor complexes; these include complementary chimeras, coimmunoprecipitation with differentially epitope-tagged receptors, the use of sodium sulphate-polyacrylamide gel electrophoresis (SDS-PAGE),



SCH 58261



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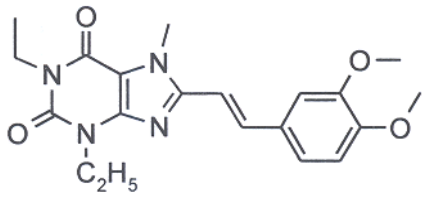

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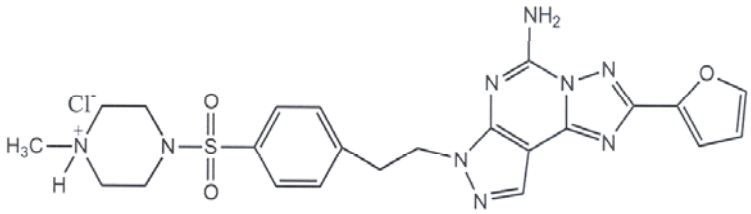

SCH BT2

Figure 2. Chemical structures of selective adenosine A_{2A} receptor antagonists. 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH 58261); (E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF 17837); (E)-1,3-dietyl-8-(3,4-dimethoxystyryl)-7-methyl-xantine (KW 6002); (2-furan-2-yl-7-3{4[4-(4-methylpiperazine-1-sulfonyl)phenylethyl}-7H-pyrazolo[4,3][1,2,4]triazolo-[1,5-c]pyrimidin-5-ylamine (SCH BT2).

often in combination with covalent cross-linking, and finally biophysical methods, namely bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET). Indeed, coimmuno-precipitation studies have demonstrated the existence of constitutive A_{2A}/D_2 complexes at colocalization sites [31,54,55]. Recently, direct physical evidence for A_{2A}/D_2 heteromers as well as A_{2A} homodimers within the plasma membrane was provided by FRET and BRET analyses, indicating less than 10 nm separating the receptors [32,56].

One functional implication of this intramembrane receptor/receptor interaction through heteromerization is the reduction of the high affinity agonist state of D_2 receptors. An additional consequence is the counteraction of

D_2 receptor G protein coupling, since A_{2A} receptor agonists counteract the GTP analog-induced appearance of D_2 receptors in the high-affinity state through an action independent of the GTP binding site [53,57]. Thus, the essence of this A_{2A}/D_2 receptor heteromerization may be to convert the D_2 receptor into a state of strongly reduced functional activity. In line with this view, A_{2A} receptor activation counteracts D_2 receptor-induced intracellular Ca^{2+} responses [30] and D_2 receptor-mediated inhibition of cAMP formation [29,31]. Moreover, evidence for functional A_{2A}/D_3 heteromers has recently been obtained in cotransfected A_{2A}/D_3 cells using FRET where A_{2A} activation reduces the affinity of D_3 agonist binding sites as well as D_3 signalling [58].

A_{2A} receptors have also been found to interact with non-dopaminergic receptors such as the mGlu5 receptor. Evidence attesting how, by combining the stimulation of A_{2A} receptors and group I mGlu, including mGlu5 receptors, the affinity of the D_2 receptor agonist binding sites in striatal membranes could be synergistically reduced [59,60] was followed by physical (coimmunoprecipitation) evidence that A_{2A} and mGlu5 receptors form heteromeric complexes [61]. These observations were supported by the high degree of A_{2A} and mGlu5 colocalization in striatal neurons in primary cultures and in glutamatergic nerve terminals in the striatum [54,62]. Coactivation of the A_{2A} and mGlu5 receptors caused a synergistic interaction at the level of c-*fos* expression and on extracellular signal-related kinase (ERK) as well on DARPP-32 phosphorylation, indicating a possible role for this heteromeric complex in striatal plasticity [61,63]. Combined A_{2A} and mGlu5 receptor activation may also produce synergistic cellular effects on striatal output neurons *in vivo*, as demonstrated by a greater than additive increase in GABA release from the ventral striatopallidal neurons after local perfusion with both A_{2A} and mGlu5 agonists [64].

The finding of heteromeric A_{2A}/D_2 complexes has added to the substantial evidence for antagonistic molecular, cellular, electrophysiological and behavioural interactions between A_{2A} and D_2 receptors, strongly supporting the logical basis for antiparkinsonian strategies that simultaneously block adenosine A_{2A} and stimulate DA (D_1 and D_2) receptors [26,65-68]. Based on these premises therefore, it is highly probable that a successful treatment with A_{2A} antagonist monotherapy in the early stages of the PD when residual endogenous DA elicits tonic D_2 receptor activation could be achieved. In the same way, the discovery of functional A_{2A}/mGlu5 receptor interactions and heteromeric A_{2A}/mGlu5 complexes supports recent data reported concerning the synergistic antiparkinsonian potential of combining A_{2A} and mGlu5 antagonists [69-71].

Parkinson's disease

PD is an age-related neurodegenerative disorder characterized by the progressive and irreversible loss of neurons from specific areas of the brain, resulting in movement deficits.

The cardinal motor features of PD are bradykinesia (slow movement and difficulty in initiation movement), muscle rigidity, resting tremor, postural instability and gait impairment [72]. These symptoms are usually manifested asymmetrically, initially affecting one body segment or half-body. Reduced facial expression, decreased eye-blinking and hypophonia are among the earlier manifestations. The onset of postural and gait disturbances usually follows this intermediate stage, often representing a major source of disability [73].

Strictly speaking, the term akinesia is used to indicate absence of movement, but in PD it usually refers to slowness in movement execution (bradykinesia) or poverty of spontaneous movements (hypokinesia). PD is characterised by a reduction in the rate and amplitude of movements. Bradykinesia may significantly impair the quality of life due to the length of time required to perform everyday tasks [74].

Rigidity is an increase in the tone of passive muscle groups that extends throughout the range of movement. This is clinically expressed as resistance in muscle groups in obtaining complete muscular relaxation. The most typical feature of rigidity is an increased resistance to passive movement of patient's limbs usually associated with a cogwheel phenomenon [75].

Tremor in PD is typically manifested at rest, tending to disappear when voluntary movement is performed. Tremor may be intermittent and is increased by stress or by manoeuvres involving concentration. [76].

Postural instability and gait disturbance appear at a later stage of the disease when postural reflexes required to maintain the centre of gravity are affected. This is associated with loss of balance and falling. As the disease progresses, freezing begins to appear. Initially, this comprises start hesitations, in which patients become frozen when trying to initiate gait, when turning or when passing through narrow spaces [73].

Non-motor features of PD abnormalities on affectivity and cognition occur frequently; patients may become passive or very quiet, with lack of initiative and slower cognitive processes. Depression can occur at any stage of the disease, whereas dementia is significantly more frequent (30%) in PD especially in older patients [77,78].

Prodromic symptoms of PD begin to emerge when there is a 40-60 % reduction of nigral neurons and striatal dopamine, but become clinically manifest when nigrostriatal DA loss reaches around 70-80 % [79,80]. In the initial stages of disease evolution, the neurodegenerative process is mainly confined to dopaminergic fibers innervating the dorsolateral putamen and, therefore, clinical manifestations primarily affect motor features. As the disease progresses, DA loss extends throughout the entire striatum, often affecting other brain regions such as the cerebral cortex, brainstem and spinal cord [73].

PD is observed in more than 1% of individuals over the age of 65 [81]. The specific aetiology of the disease is mainly unknown and the manifestation

of symptoms probably stems from both genetic and environmental abnormalities (vulnerability factor) and epigenetic stimuli such as oxidative stress, mitochondrial dysfunction and excitotoxicity, which are normally tolerated but that might become particularly harmful in predisposed individuals causing the specific deterioration of the nigrostriatal dopaminergic pathway [82,83].

The pathogenesis of PD stems from the progressive preferential degeneration of melanized neurons of the SNc which innervate the striatum. In addition to the degeneration of dopaminergic neurons, the formation of inclusion bodies of proteins aggregates (Lewy bodies) represents a peculiar consistent hallmark of the disease [84]. Changes in the SNc are most pronounced in the ventro-lateral region. Neurodegenerative modifications can also be detected in the locus ceruleus, nucleus basalis of Meynert, peducolopontine nucleus (PPN), cerebral cortex and spinal cord. The biochemical peculiarity of PD comprises a reduction of striatal DA with changes being most pronounced in the postero-lateral putamen, region that receives innervation from the ventro lateral part of the SNc [85]. Changes in serotoninergic, noradrenergic, glutamatergic and cholinergic neurotransmitter can also be observed.

Briefly, the DA deficiency in CPu reduces activation of DA receptors resulting in attenuated inhibition of the indirect pathway neurons and in a decreased excitation of the direct pathway neurons. The reduced inhibition of the indirect pathway leads to disinhibition of the STN and increased excitation of the GPi/SNr neurons, whereas decreased activation of the direct pathway causes a reduction in its inhibitory influence on the GPi/SNr. The net result is an excessive activation of the BG output neurons accompanied by excessive inhibition of motor systems, leading to parkinsonian motor features [73]. Moreover, DA depletion induces many secondary changes which affect neuronal loops connecting the BG, thalamus and cortex, including the glutamate-induced overexcitation of neurons localized in the STN and projecting to SNr/GPi [73,86]. This leads to an increase in the activity of the nigro/GPi GABAergic pathway [20,73] which inhibits the thalamus and PPN. Finally, inhibition of the PPN induces abnormal behaviour of spinal neurons which participate in increased muscle tone [75,87].

Pharmacological treatment of parkinsonian motor disabilities and the therapeutic potential of A_{2A} receptor antagonists

Since parkinsonian motor symptoms occur as a consequence of DA depletion in the BG [88], DA replacement strategies represent the main therapeutic approach used to counteract PD motor impairment: consequently, the DA metabolic precursor L-DOPA (L-3,4-dihydroxyphenilalanine) is currently the most widely used and effective antiparkinsonian drug. Enzymatic decarboxylation converts L-DOPA into DA which, by acting on dopaminergic receptors in the BG, facilitates movement initiation and execution [89], thus

reducing PD motor disabilities. Even though L-DOPA is considered the "gold standard" treatment for PD, its use is not devoid of disadvantages which reduce the therapeutic potential of the drug [90-92].

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, a condition commonly reported as "wearing off". 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 are once again manifested. Moreover, prolonged use of L-DOPA is associated with the onset of dyskinesias and abnormal involuntary movements, which are highly disabling for patients, and with fluctuations of L-DOPA motor effects, also known as "on/off" phenomenon. During "on/off" phenomena patients may rapidly pass from a condition in which L-DOPA has no effect to one in which L-DOPA counteracts PD symptoms whilst at the same time inducing dyskinesias and abnormal involuntary movements. Finally, the increase of both peripheral and central DA levels produced by L-DOPA may induce adverse effects such as nausea, orthostatic hypotension, hallucinations and mental confusion [93,94].

On the basis of the above issues, it is commonly accepted that L-DOPA should be used sparingly in order to limit its side effects and delay the onset of "wearing off" and "on/off" phenomena, as well as of dyskinesias. Thus, L-DOPA is often administered in combination with other drugs, such as direct DA agonists, anticholinergics and agents acting on DA catabolism (MAO-B and COMT inhibitors), to reduce the dose of L-DOPA capable of relieving PD motor impairment. However, the majority of drugs currently associated to L-DOPA are characterised by either an unsatisfactory efficacy on parkinsonian motor symptoms, often limited to the early stages of the disease, or several adverse effects limiting their therapeutic use. Therefore, particular effort has been put into the discovery of new drugs to be used, as monotherapy or in combination with L-DOPA, in PD treatment.

Over recent years, adenosine A_{2A} receptor antagonists have emerged among new drugs as the best candidates for PD therapy. As described above, adenosine extensively interacts with dopaminergic transmission [95-97] modulating the genesis and expression of movement [89], and experimental evidence strongly accounts for a beneficial effect of A_{2A} antagonists on PD symptoms. Moreover, the brain localization of A_{2A} receptors restricted to the BG would result in a reduced incidence of non-motor central effects by A_{2A} blockade. In addition, A_{2A} antagonists have been shown not to exert excessive peripheral adverse effects, being generally well tolerated [98]. Another feature which renders these drugs very attractive as possible antiparkinsonian agents is their neuroprotective potential, which has been demonstrated in rodent models of PD neurodegeneration and seems to suggest a preventive action for A_{2A} antagonists on PD onset and development [99-101].

The structures of the most widely used A_{2A} antagonists are reported in Figure 2.

The remaining part of this chapter will summarize the motor effects of A_{2A} antagonists observed in animal models of PD as well as in clinical trials performed to date. Moreover, data demonstrating the protective effect of A_{2A} antagonists on animal models of parkinsonian neuronal degeneration will be reported.

Effects of adenosine A_{2A} antagonists in animal models of Parkinson's disease

Acute effects on motor disability: Animal models

The study of the behavioural effects of adenosinergic ligands in experimental rodents has provided the first evidence that adenosine is involved in the control of movement. Several reports have shown that non selective adenosine blockers, like caffeine and theophylline, stimulate motor activity in rats and mice [102,103], an effect that has been demonstrated to be mainly a consequence of A_{2A} receptor blockade [104,105]. The crucial role of A_{2A} receptor in the modulation of motor behaviour has been further confirmed by studies employing selective ligands towards this receptor. In particular, inhibition of motor behaviour has been observed following administration of the A_{2A} agonist CGS 21680 [106-108], whereas the A_{2A} antagonist SCH 58261 was found to stimulate motor activity [42,47,109]. Interestingly, a beneficial effect on motor impairment by A_{2A} receptor blockade has been demonstrated in several animal models of PD, including counteraction of catalepsy and modulation of rotational behaviour in rodents, as well as reversion of motor impairment in primates (Tab. 1).

Catalepsy is a condition commonly induced in rats and mice by administration of different pharmacological agents, such as neuroleptics, cholinomimetics or the monoamine-depleting agent reserpine. Catalepsy is characterized by akinesia associated to a hypofunctionality of the striatal DA system, which is believed to mimic that observed in PD [110]. Drugs commonly used in PD treatment are known to counteract catalepsy [111,112], therefore reversion of catalepsy can be considered as a valuable experimental tool to investigate the antiparkinsonian properties of new drugs.

A wide number of A_{2A} receptor antagonists have been demonstrated to effectively counteract catalepsy in rodents, reducing its severity and duration, thus accounting for an amelioration of parkinsonian motor impairment by these drugs [113-116] (Tab. 1). Interestingly, potentiation of the anticataleptic effect of L-DOPA has been described following its coadministration with the A_{2A} antagonists KF 17837 and KW 6002 [113,114], indicating the existence of a synergistic interaction between L-DOPA and A_{2A} antagonists (Fig. 2 and Tab. 1).

Table 1. Summary of the effects of A_{2A} antagonists in animal models of Parkinson's disease.

Experimental Model	Effects
Rat 6-OHDA lesion (acute) [122-125]	✓ Potentiation of contralateral rotational behaviour stimulated by L-DOPA and DA agonists
Rat haloperidol-induced catalepsy (acute) [113-116]	✓ Reversal of catalepsy ✓ Potentiation of L-DOPA anticataleptic effect
Rat tremulous jaw movements (acute) [166,167]	✓ Counteraction of parkinsonian-like tremor
Rat haloperidol-induced muscle rigidity (acute) [171,172]	✓ Amelioration of parkinsonian-like muscle rigidity
Primate MPTP lesion (acute) [131-133]	✓ Reversal of motor disability ✓ Amplification of L-DOPA beneficial effects on motor impairment
Rat 6-OHDA lesion (chronic) [147,151]	✓ No sensitization to L-DOPA-induced contralateral rotational behaviour ✓ Reversal of the progressive shortening of L-DOPA-induced contralateral rotational behaviour
Primate MPTP lesion (chronic) [131-133,151]	✓ Lack of dyskinetic effects ✓ Lack of worsening of L-DOPA-induced dyskinesia ✓ Delay of apomorphine dyskinetic effects
Early-genes in 6-OHDA-lesioned rats (acute) [122-124]	✓ Potentiation of *c-fos* expression stimulated by L-DOPA and DA agonists
Peptides and GAD 67 mRNA in 6-OHDA–lesioned rats (chronic) [158,159]	✓ Attenuation in the increase of enkephalin, dynorphin and GAD 67 mRNA compared to L-DOPA full dose
Receptors in 6-OHDA-lesioned rats (chronic) [151,161]	✓ Reduction of L-DOPA-stimulated phosphorylation of AMPA receptors
Neuronal survival and DA levels MPTP treated mice (acute and chronic) and in 6-OHDA-lesioned rats (acute) [99,195-198]	✓ Reduced parkinsonian-like degeneration of dopamine SNc neurons ✓ Attenuation of the decrease in striatal DA contents

In agreement with observations made in the catalepsy model, A_{2A} receptor antagonists displayed motor facilitatory activity in animals rendered parkinsonian by the administration of dopaminergic neurotoxins, like 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Such neurotoxins

produce a degeneration of mesencephalic dopaminergic neurons, mimicking that occurring in idiopathic PD and resulting in the presence of parkinsonian-like symptoms (akinesia, bradykinesia etc.) in the treated animals, thus providing valuable and reliable PD models [110,117].

The rat 6-OHDA model is induced by the unilateral medial forebrain bundle or SNc infusion of this neurotoxin leading to a functional unbalance of the dopaminergic striatal terminal and, consequently, to unilateral motor impairment which is typically expressed by rotational behaviour upon administration of dopaminomimetic drugs [118,119]. Indeed, it is well established that the ability of a specific drug to induce contraversive rotational behaviour, as well as to potentiate the rotational behaviour stimulated by DA receptor agonists, can be assumed to be a parameter reflecting its antiparkinsonian activity [117,120].

Acute administration of the A_{2A} agonist CGS 21680 to unilaterally 6-OHDA-lesioned rats has been demonstrated to significantly reduce rotational behaviour induced by L-DOPA and by either D_1 or D_2 DA receptor agonists [121]. In contrast, acute administration of the A_{2A} antagonist SCH 58261 to 6-OHDA-lesioned rats has been shown to significantly potentiate rotational behaviour induced by L-DOPA and by either D_1 or D_2 DA receptor agonists [122-124] (Tab. 1). Similarly, an enhancement of rotational behaviour stimulated by L-DOPA or apomorphine was observed following acute A_{2A} receptor blockade by KF 17837 or KW 6002 [125] (Tab. 1). Behavioural findings have been supported by neurochemical data showing an enhanced responsiveness to dopaminergic stimuli by striatal neurons. In particular, acute SCH 58261 administration amplified the expression of the immediate early gene *c-fos*, an index of neuronal activation [126], stimulated in the dopamine-denervated striatum by L-DOPA as well as direct DA agonists [122-124] (Tab. 1).

Reversal of motor impairment by A_{2A} antagonists has also been observed in MPTP-treated non human primates. MPTP-induced parkinsonism is considered as the best animal model of PD, due to the anatomical and functional similarities existing between dopaminergic neurodegeneration observed in idiopathic PD and MPTP intoxication [127-129]. Furthermore, non human primates treated with MPTP display a large number of motor symptoms which accurately mimic those typical of human PD [130].

The A_{2A} antagonist KW 6002 acutely increased locomotor activity and reversed motor disabilities in common marmosets and cynomolgus monkeys previously administered with MPTP [131-133] (Tab. 1). Moreover, similar to findings reported in other animal models of PD, a synergistic interaction between A_{2A} antagonists and L-DOPA, as well as dopaminergic agonists, on motor disability has been observed in MPTP-treated common marmosets [132] (Tab. 1).

Regarding the mechanism whereby A_{2A} antagonists counteract motor impairment in PD animal models, solid evidence accounts for a pivotal role for the opposite interactions between A_{2A} and D_2 receptors. Thus, A_{2A} receptor

antagonists, by acting at striatopallidal neuronal level, enhance D_2-mediated effects. Moreover, A_{2A} receptor blockade indirectly amplifies the effects produced by stimulation of D_1 receptors located in the striatonigral neurons [122,123]. Thus, the facilitatory effect produced by A_{2A} antagonists on DA transmission may result in motor stimulation, explaining the beneficial activity of these drugs on parkinsonian-like motor impairment.

The finding that the motor effects of A_{2A} antagonists are at least in part independent of D_2 receptors [50] suggests that alternative mechanisms, in addition to the interaction between A_{2A} and DA receptors might be involved in the motor facilitating activity of A_{2A} antagonists. Indeed, blockade of A_{2A} receptors located on cholinergic interneurons has been shown to reduce the efflux of acetylcholine in the striatum [134,135], whereas electrophysiological data indicate that A_{2A} receptors modulate GABA release from axon recurrent collaterals and terminals of striatopallidal neurons, regulating the activity of the striatopallidal pathway [136,137]. This effect has been suggested to be implicated in the motor facilitating effects of A_{2A} antagonists [138], since an altered functionality of the striatopallidal pathway contributes to the production of PD motor symptoms [139,140]. Furthermore, interactions between A_{2A} and mGlu5 receptors have also been implicated in the effects of A_{2A} antagonists in PD animal models [69,71].

On the basis of the highly selective expression of A_{2A} receptors on striatopallidal neurons [21], the striatum is considered the main locus in which A_{2A} antagonists counteract PD motor impairment (Fig. 1). The crucial role of the striatum in the effects of A_{2A} antagonists has been confirmed by studies showing that the intrastriatal infusion of the A_{2A} antagonist MSX-3 effectively reversed catalepsy induced by D_1 or D_2 receptor antagonists [141]. Interestingly, recent evidence obtained in 6-OHDA-lesioned rats has demonstrated a potentiation of L-DOPA-induced rotational behaviour following infusion of the A_{2A} antagonist SCH BT2 [142] (Fig. 2) into the GP, the output nucleus of the striatopallidal neurons [143]. This latter finding is of particular interest due to the implication that besides the well documented role of the striatum in mediating motor facilitation produced by A_{2A} antagonists, extrastriatal circuits may also be involved in this effect.

To summarize, findings obtained in animal models of PD provide strong indications that A_{2A} antagonists exert antiparkinsonian activity. Notably, synergistic interactions between L-DOPA and A_{2A} antagonists have been described in different experimental paradigms, suggesting that these drugs might potentially be coadministered with L-DOPA in order to potentiate its motor stimulant effects. Furthermore, the results about the mechanisms and sites of action suggest that A_{2A} antagonists modulate BG functionality in a wide and complex fashion. In particular, evidence postulating the influence exerted by A_{2A} receptors on neurotransmitters such as acetylcholine, GABA

and glutamate, which are critically involved in the pathogenesis of PD [144-146], is of great interest in the light of new therapeutic approaches employing A_{2A} antagonists in combination with drugs acting on the above neurotransmitters in counteracting PD.

Chronic effects on motor disability and on dyskinesia: Animal models

Consistent with findings reported following acute administration, chronic A_{2A} antagonists have been demonstrated to effectively ameliorate motor deficits in parkinsonian rats and primates and, in addition, to not produce tolerance to their motor stimulant effects (Tab. 1).

The persistence of the motor effects of A_{2A} antagonists has been described in both rats and primates chronically administered with these drugs. In 6-OHDA-lesioned rats, the potentiation of the intensity of L-DOPA-induced rotational behaviour elicited by acute SCH 58261 has been observed even after a prolonged administration regimen (up to 14 days) of the A_{2A} antagonist [147]. Accordingly, in MPTP-treated common marmosets chronic (21 days) treatment with KW 6002 produced a relief of parkinsonian motor disability with no sign of tolerance to this effect [131].

Tolerance is usually observed in rodents following long-term exposure to non selective adenosinergic blockers like caffeine and theophylline which act on both A_1 and A_{2A} receptors [102,148]. Interestingly, cross-tolerance between caffeine and the selective A_1 receptor antagonist CPT has been reported, whereas no changes in the motor stimulant effects of the A_{2A} antagonist MSX-3 have been described in caffeine-tolerant rats [108]. Thus, it has been suggested that A_1, but not A_{2A}, receptors are increasingly involved in the development of tolerance to adenosinergic ligands, thereby justifying the results observed in parkinsonian animals chronically administered with A_{2A} antagonists. The absence of tolerance to the motor stimulant effects of A_{2A} antagonists is of paramount interest in a condition requiring a long-term pharmacological treatment such as PD, in which drugs capable of retaining their motor facilitating properties over a chronic administration regimen are required.

A major outcome emerging from studies on chronic A_{2A} antagonists is represented by results reported with regard to motor fluctuations and dyskinesia in animals coadministered with A_{2A} antagonists and L-DOPA.

Studies in 6-OHDA-lesioned rats have shown that the duration of rotational behaviour elicited by L-DOPA is progressively reduced during chronic administration of the drug, a phenomenon that is thought to reproduce the L-DOPA "wearing off" observed in humans [149,150]. Coadministration of the A_{2A} antagonist KW 6002 prevented the shortening of rotational behaviour, reflecting a potential beneficial influence of A_{2A} blockade on L-DOPA "wearing off" [151] (Tab. 1). Despite the reduced duration, the

intensity of rotational behaviour induced by L-DOPA in 6-OHDA-lesioned rats undergoes a progressive increase (sensitization) during long-term administration. Sensitization of rotational behaviour is thought to mimic dyskinetic effects elicited by L-DOPA, representing a rat model of L-DOPA-induced dyskinesia [152,153]. In this paradigm, interesting results concerning the modulation of dyskinesias by A_{2A} receptor blockade have been obtained comparing the rotational behaviour elicited by long-term administration of a higher dose of L-DOPA with an equipotent combination of a lower dose of L-DOPA plus the A_{2A} antagonist SCH 58261. Although both L-DOPA (high dose) and L-DOPA (lower dose) plus SCH 58261 produced a comparable degree of rotations on the first administration, sensitization of rotational behaviour was observed in response to chronic L-DOPA alone but not to chronic L-DOPA plus SCH 58261 [147] (Tab. 1). The stable response observed after long-term L-DOPA plus SCH 58261 suggests that the association between the two drugs represents a treatment with low dyskinetic potential. Interestingly, this hypothesis has been strengthened by studies showing that genetic deletion of the A_{2A} receptor prevents the sensitization of rotational behaviour stimulated by L-DOPA in 6-OHDA-lesioned mice [154].

The results obtained in MPTP-treated primates confirm and further extend those deriving from 6-OHDA-lesioned rats. First, A_{2A} antagonists do not induce dyskinesia *per se*, since administration of KW 6002 to parkinsonian primates relieved motor disability without stimulating abnormal movements [131,133] (Tab. 1). Second, in MPTP-treated marmosets previously exposed to chronic L-DOPA in order to develop dyskinesia, motor stimulation induced by KW 6002 was not associated with an exacerbation of dyskinetic movements [131] (Tab. 1). Furthermore, no sign of apomorphine-induced dyskinesia has been observed in parkinsonian cynomolgus monkeys chronically treated with a combination of apomorphine and KW 6002 [151]. Interestingly, if KW 6002, but not apomorphine, administration was interrupted, primates previously treated with KW 6002 displayed apomorphine-induced dyskinesia only 10-12 days after KW 6002 discontinuation, thus accounting for a potential preventive effect of A_{2A} receptor blockade on the development of dyskinesia [151] (Tab. 1).

Despite the effects reported on dyskinesia onset in rats and primates, it should be noted that no study has yet demonstrated the ability of A_{2A} antagonists to revert an already established dyskinesia in animal models. Nevertheless, experimental evidence suggests that A_{2A} antagonists associated with a low non-dyskinetic dosage of L-DOPA may achieve satisfactory results in motor stimulation, whilst at the same time limiting the severity of L-DOPA-induced dyskinesia. It has been shown that in MPTP-treated common marmosets previously rendered dyskinetic by chronic L-DOPA, the relief of motor impairment produced by an optimal dose of L-DOPA presenting a high dyskinetic potential was adequately mimicked by the combination of KW 6002

plus a suboptimal dose of L-DOPA which, on the contrary, was associated with weak induction of dyskinesia [132]. Interestingly, no worsening of dyskinetic movements was observed over a 5 day treatment period with KW 6002 plus L-DOPA, further supporting a potential palliative effect of A_{2A} antagonists in the treatment of established dyskinesias (Tab. 1).

The study of the effects of A_{2A} antagonists on behavioural parameters in both rat and primate dyskinesia models has been paralleled by the analysis of the influence of A_{2A} blockade on the biochemical modifications induced by chronic L-DOPA in the BG of 6-OHDA-lesioned rats (Tab. 1). Prolonged administration of L-DOPA, according to a regimen capable of inducing a sensitized (dyskinetic-like) rotational response, has been shown to modify the expression of the neuropeptides ENK and DYN as well as of the enzyme glutamic acid decarboxylase (GAD67) in the BG of 6-OHDA-lesioned rats [155-159]. Thus, although a direct relationship between these biochemical changes and L-DOPA-induced dyskinesia onset has not been univocally demonstrated, they have nevertheless been postulated to reflect a more general aberrant functionality of BG produced by long-term L-DOPA, which is thought to underlie the dyskinesia elicited by this drug [160].

Coadministration of low doses of L-DOPA with the A_{2A} antagonist SCH 58261 did not induce modifications in the striatal levels of ENK, DYN and GAD67 produced by chronic higher doses of L-DOPA in 6-OHDA-lesioned rats [158] (Tab. 1). Similarly, no changes in GAD67 levels were induced by chronic SCH 58261 plus L-DOPA in the GP [159].

Moreover, beneficial effects of A_{2A} blockade on the regulation of the phosphorylation state of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type of glutamate receptors by L-DOPA have been described. Chronic administration of L-DOPA to 6-OHDA-lesioned rats leads to hyperphosphorilation of the striatal AMPA receptor, an effect that has been postulated to participate in the genesis of L-DOPA-induced dyskinesias, being prevented by co administration of KW 6002 [151,161] (Tab. 1). Taken together, these biochemical outcomes indicate that A_{2A} blockade extensively regulates the functionality of BG circuits and modulates the neuronal responsiveness to L-DOPA, suggesting that these actions might be involved in the beneficial behavioural effects of A_{2A} antagonists observed in animal models of dyskinesia.

The neuronal mechanisms underlying the regulation of BG activity by A_{2A} antagonists might involve both post- and presynaptic A_{2A} receptors. Thus, modulation of striatal neuronal functionality by regulation of postsynaptic receptor and kinases [162] or the modulation of neurotransmitter release [25], might influence BG neuroplasticity as well and, in turn, the onset of dyskinesia.

In addition to the postulated A_{2A} receptor regulatory effects on neuronal responsiveness following prolonged dopaminergic stimuli, it should be considered that A_{2A} antagonists, by potentiating the motor effects of L-DOPA

or dopaminergic drugs allow the latter to be used at low non-dyskinetic doses [124,125,132,147]. Therefore, the sparing of dopaminomimetic agents produced by coadministration with A_{2A} antagonists might contribute towards reducing, or at least delaying, the onset of neuroplastic modifications in the BG, thus enabling maintenance therapy with the above drugs at doses presenting a reduced incidence of dyskinetic effects.

Taken together, data obtained from several preclinical studies point to the existence of beneficial effects for chronic A_{2A} antagonists on PD motor disability and on motor complications produced by long-term L-DOPA. These effects are of considerable interest in light of the fact that motor complications are one of the intrinsic limits of L-DOPA therapy and are often insensitive to pharmacological manipulation. Moreover, lack of tolerance to the motor effects of A_{2A} antagonists suggests that these drugs can be successfully used over long periods, rendering them particularly suitable for a prolonged pharmacological treatment such as that required in PD.

Effects on parkinsonian tremor and muscle rigidity: Animal models

Resting tremor is one of the earliest symptoms manifested by parkinsonian patients and, interestingly, recent studies have indicated that A_{2A} antagonists exert beneficial effects in rat models of parkinsonian tremor.

The antitremorigenic properties of drugs can be evaluated in experimental animals by measuring their ability in counteracting tremulous jaw movements induced in rats by a wide number of pharmacological agents, ranging from the DA receptor antagonist haloperidol to cholinomimetic drugs, such as tacrine or pilocarpine [163]. Although human parkinsonian resting tremor mainly affects the hands and is less frequently observed in the jaw, rat tremulous jaw movements have been shown to share many electromyographic and pharmacological characteristics with parkinsonian tremor, including frequency (3-7 Hz), responsiveness to common antiparkinsonian drugs and striatal origin [163-165].

Acute administration of the A_{2A} antagonist SCH 58261 reversed jaw tremor stimulated by tacrine in rats [166] and, accordingly A_{2A} blockade by KF 17837 was found to effectively counteract tremulous jaw movements induced by haloperidol [167] (Tab. 1). Moreover, analysis of the striatal sites involved in the counteraction of tremor by A_{2A} antagonists has evidenced a critical role for the ventrolateral striatum in this effect. Thus, an almost complete suppression of tacrine-induced tremulous jaw movements has been observed following the infusion of the A_{2A} antagonist SCH BT2 into the ventrolateral striatum, whereas only a partial effect was produced if SCH BT2 was infused into the dorsomedial striatum [166]. Notably, rat tremulous jaw movements have been shown to originate mainly at the level of the ventrolateral striatum [163], therefore the site-dependence of the antitremorigenic effects of A_{2A}

antagonists likely reflects a direct suppressive effect of these drugs on the genesis of tremor.

On the basis that striatal acetylcholine plays a critical role in promoting the genesis of tremulous jaw movements [163], it has been hypothesised that modulation of cholinergic transmission by A_{2A} antagonists might underlie the antitremorigenic effects of these drugs observed in rats. The postulated interactions between A_{2A} antagonists and acetylcholine occur mainly at the presynaptic level, where blockade of A_{2A} receptors located on striatal cholinergic interneurons is known to reduce acetylcholine outflow [134,135]. On the other hand, postsynaptic cholinergic mechanisms do not seem to be involved, since blockade of A_{2A} receptors by SCH 58261 was found to be ineffective in reversing jaw tremor stimulated by the direct muscarinic agonist pilocarpine [168]. However, since several other neurotransmitters, including DA, GABA and glutamate, are known to regulate the expression of tremulous jaw movements in rats [163,169], interactions between A_{2A} receptors and DA, as well as the influence of A_{2A} antagonists on neurotransmitters other than acetylcholine and DA might be involved in the antitremorigenic effect of these drugs.

Muscle rigidity is a characteristic symptom of PD, in addition to tremor and akinesia. Administration of adequate doses of haloperidol or reserpine to rats induces muscle rigidity sharing many mechano- and electromyographic features with that observed in PD patients [170]. Acute blockade of A_{2A} receptors by SCH 58261 was found to effectively counteract parkinsonian-like muscle rigidity in rats, affecting both mechano- and electromyographic components of the rigidity [171] (Tab. 1), an effect that has been proposed to be mediated by the facilitation of DA transmission at the postsynaptic level [171,172].

Taken together, the effects of A_{2A} antagonists obtained in PD animal models indicate that these drugs not only ameliorate motor impairment, but also effectively counteract parkinsonian-like resting tremor and muscle rigidity. Notably, these latter effects are of particular interest for a potential clinical use of A_{2A} antagonists since, in parkinsonian patients, resting tremor and muscle rigidity are often insensitive to classic antiparkinsonian medications.

Effects of adenosine A_{2A} antagonists in parkinsonian patients: Results from clinical trials

A first evaluation of the potential antiparkinsonian effect produced in humans by the manipulation of adenosine receptors has been performed using the non specific antagonists theophylline and caffeine. The results obtained in these early clinical trials, although evidencing several positive effects, were however contradictory.

Long-term administration of theophylline was found to ameliorate parkinsonian motor disability and to counteract resting tremor [173,174]. In patients with advanced PD, coadministration of theophylline has been reported to extend the duration of the L-DOPA "on" period [175], but this result has not yet been definitively confirmed [176]. Moreover, in both these latter reports, a worsening, rather than improvement, of resting tremor was described following theophylline administration [175,176]. In a similar way, clinical evaluation of caffeine did not definitively demonstrate an antiparkinsonian effect for this drug [177,178]. However, it should be noted that clinical trials have often employed high doses of either caffeine or theophylline which are known to induce motor depression, rather than stimulation [179,180]. Therefore, on one hand early clinical trials suggested the existence of potential beneficial effects by adenosine receptor blockade in parkinsonian patients, whilst on the other, underlined the need to use more effective specific compounds.

The development of new selective antagonists towards A_{2A} receptors and their encouraging effects in animal models of PD have provided a rationale for the re-evaluation of adenosine antagonists as potential antiparkinsonian drugs in humans. Accordingly, the large body of evidence available on the effects of A_{2A} antagonists in PD patients has been obtained using the compound KW 6002 (also called istradefylline), currently in stage III of clinical evaluation. Results obtained in patients treated with KW 6002 largely confirm findings observed following administration of A_{2A} antagonists to parkinsonian rodents and primates, although discrepancies have been reported [101,181].

In an initial trial, the effects of KW 6002, given either alone or in combination with intravenous L-DOPA, have been evaluated in patients with advanced PD [161,182]. When administered as monotherapy, KW 6002 neither improved motor impairment nor induced dyskinesias per se. Moreover, coadministration of KW 6002 did not modify the motor effects and dyskinesia stimulated by an optimal dose of L-DOPA, although a longer duration of the L-DOPA "on" phase was described [98]. On the other hand, combined administration of KW 6002 plus a suboptimal dose of L-DOPA resulted in a significant improvement of motor disability comparable to that produced by an optimal dose of L-DOPA, being however associated with a lesser degree of dyskinesias [161]. The combination of KW 6002 plus the low dose of L-DOPA ameliorated all cardinal symptoms of PD, in particular resting tremor [161,182]. In agreement with the results obtained using KW 6002, potentiation of the effects of L-DOPA has recently been reported in PD patients following coadministration with the novel A_{2A} antagonist SCH 420814 [183].

The ineffectiveness of KW 6002 monotherapy in counteracting parkinsonian symptoms in humans is at variance with results obtained in animals, in which KW 6002 reverted motor disability when given alone [114,131-133]. A possible explanation for this discrepancy may depend on the

fact that the doses used in clinical trials have been too low and that higher doses of KW 6002 could exert antiparkinsonian activity as a monotherapy. Moreover, it should be taken into account that clinical trials have been performed in patients with advanced PD, presumably associated with considerable dopaminergic degeneration. Therefore, it may be hypothesised that residual endogenous DA might be fundamental in obtaining a complete expression of the antiparkinsonian effects produced by A_{2A} receptor blockade, thus justifying the ineffectiveness of KW 6002 when administered alone.

A major finding emerging from the clinical evaluation of KW 6002 is represented by its ability to extend the duration of L-DOPA "on" phase [98,182]. Further studies in patients experiencing L-DOPA motor fluctuations have confirmed this result, showing however that the effect was coupled with an enhancement in L-DOPA-induced dyskinesias [98,184,185]. This latter finding appears to contradict primate studies in which KW 6002 was found to potentiate the effects of L-DOPA without exacerbating dyskinesia [132]. Nevertheless, a close examination of the characteristic features of dyskinesia has highlighted how KW 6002 prolonged the duration of the "on" phase associated with the so called "non-troublesome" dyskinesia, whereas the duration of the "on" phase associated with "troublesome" dyskinesia was unaltered following KW 6002. Furthermore, the overall severity of L-DOPA-induced dyskinesia was not exacerbated by KW 6002 [98]. Therefore, the enhancement of dyskinesia produced by KW 6002 does not seem to preclude the use of either this drug or, generally, of other A_{2A} antagonists in human PD treatment.

In addition to beneficial motor effects, clinical trials have demonstrated that KW 6002 is well tolerated, the only adverse effects reported being nausea, increased stiffness and headache, which however were reported to be of mild intensity and which resolved spontaneously during treatment [98,184]. Similar results have been reported for the novel A_{2A} antagonists SCH 420814 [183] suggesting that this class of drugs is suited for use in long-term pharmacological treatment.

Taken together, the outcomes deriving from clinical trials performed in humans illustrate an encouraging therapeutic potential for A_{2A} antagonists in the treatment of PD. Indeed, the observed temporal extension of the L-DOPA "on" phase is of particular interest, since the progressive shortening of L-DOPA motor effects ("wearing off") represents one of the main inconveniences manifested during prolonged use of this drug. Nevertheless, further studies involving early stage parkinsonian patients are required to clarify whether A_{2A} antagonists are suited for use in monotherapy. Furthermore, assessment of these drugs in patients devoid of L-DOPA motor complications should be performed in order to evaluate in detail the effect of A_{2A} antagonists on human dyskinesia onset and development.

Potential neuroprotective effect of A_{2A} antagonists in Parkinson's disease

One of the major limitations of the current pharmacological treatment of PD is represented by its substantial ineffectiveness in counteracting the degeneration of dopaminergic neurons which underlies this condition, therefore the search for therapeutic strategies capable of arresting, or at least slowing down, this process is of paramount importance. Consequently, it has recently been emphasised that the blockade of adenosine A_{2A} receptors might potentially represent a valuable approach in counteracting neuronal death in PD.

Previous studies have proposed that, in the brain, adenosine A_{2A} receptors critically regulate neuronal survival in response to neurotoxic insults [186,187], ranging from ischemia [188-190] to excitotoxicity [191,192].

Notably, neuroprotection by A_{2A} receptor blockade has also been described in rodent models of neurodegenerative diseases. Administration of A_{2A} antagonists has been shown to counteract striatal degeneration in a rat model of Huntington's disease [193] and to prevent β-amyloid-induced neurotoxicity in cultures of rat cerebellar cells, a putative model of Alzheimer's disease [194]. Moreover, interesting results demonstrating neuroprotective effects by A_{2A} blockade have been obtained in the mouse model of MPTP-induced parkinsonian-like neurodegeneration. In this paradigm, administration of the non selective adenosine antagonist caffeine or of specific A_{2A}, but not A_1, antagonists was found to elicit robust neuroprotection, preventing the loss of striatal DA and of nigral dopaminergic neurons induced by MPTP [99,195-197] (Tab. 1). Consistently, neuroprotective effects of A_{2A} antagonists have been described in complementary experiments using the 6-OHDA rat model of dopaminergic neurodegeneration [198] (Tab. 1). Notably, data deriving from pharmacological studies have been substantiated by genetic experiments demonstrating a prevention of MPTP-induced neurotoxicity by the deletion of the A_{2A} receptor in mice [99].

The mechanisms by which A_{2A} receptor blockade elicits protective effects in models of PD neurotoxicity are not fully known; however, experimental evidence has suggested that glutamate might be involved in this effect [101,187], being considered a facilitator of the neurotoxic dysfunctions that form the base for PD neurodegeneration [199-201]. Interestingly, A_{2A} receptors, located on either presynaptic glutamatergic terminals or glial cells [10,11,18], have been shown to modulate glutamate extracellular concentrations extensively in several brain areas including BG. In particular, stimulation of A_{2A} receptors enhances glutamate release which, in contrast, is reduced following A_{2A} blockade [202,203]. Based on the above, it has been hypothesised that A_{2A} receptor blockade might result in a relief of glutamatergic output to DA SNc neurons and, in turn, in a reduction of PD

neurodegeneration [187]. Nevertheless, in view of the large number of neuronal functions regulated by A_{2A} receptors [25], it is possible to maintain that mechanisms other than modulation of glutamate release might be involved in the neuroprotective effect of A_{2A} antagonists.

To date, no studies have been performed to evaluate the exerting of beneficial effects by A_{2A} antagonists in human PD neurodegeneration. Nevertheless, the protective effects of A_{2A} receptor blockade observed in animal models of parkinsonian-like neurotoxicity are of particular interest in light of epidemiological research demonstrating the existence of an inverse relationship between caffeine consumption and incidence of PD [204,205]. Indeed, although these studies have not demonstrated the ability of caffeine to prevent the onset of PD conclusively [101], it is conceivable that the inverse relationship between caffeine consumption and PD might be due to neuroprotective effects on dopaminergic mesencephalic neurons subsequent to persistent A_{2A} receptor blockade by caffeine. Therefore, direct evidence of neuroprotection mediated by A_{2A} receptor antagonists in animals, as well as data from epidemiological studies, provide new insights into the study of the antiparkinsonian potential of these drugs. It can therefore be postulated that A_{2A} receptor antagonists might not only relieve motor deficits in established PD, but also potentially prevent the progress of the pathology by arresting degeneration of dopaminergic mesencephalic neurons.

Although neuroprotective effects represent an intriguing feature of the antiparkinsonian activity of A_{2A} antagonists, it should however be highlighted that A_{2A} receptor blockade might produce side effects outside the brain. Indeed, stimulation of A_{2A} receptors has been demonstrated to reduce renal and cardiac damage during ischemia [206-208] and to attenuate inflammatory processes [209,210]. Moreover, neuroprotective effects have been observed in the spinal cord following A_{2A} receptor stimulation [211], thus indirectly accounting for potential negative effects of A_{2A} blockade at this level. Further studies should be carried out both in animals and humans in order to evaluate all the possible implications of A_{2A} receptor blockade, with the specific aim of clarifying whether, and under which circumstances, A_{2A} antagonists might be used as neuroprotective agents in PD patients.

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