Published in final edited form as:
Pharmacol Ther. 2011 December ; 132(3): 280–299. doi:10.1016/j.pharmthera.2011.07.004.

Past, present and future of A_{2A} adenosine receptor antagonists in the therapy of Parkinson's disease

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Abstract

Several selective antagonists for adenosine A_{2A} receptors ($A_{2A}R$) are currently under evaluation in clinical trials (phases I to III) to treat Parkinson's disease, and they will probably soon reach the market. The usefulness of these antagonists has been deduced from studies demonstrating functional interactions between dopamine D_2 and adenosine A_{2A} receptors in the basal ganglia. At present it is believed that $A_{2A}R$ antagonists can be used in combination with the dopamine precursor L-DOPA to minimize the motor symptoms of Parkinson's patients. However, a considerable body of data indicates that in addition to ameliorating motor symptoms, adenosine $A_{2A}R$ antagonists may also prevent neurodegeneration. Despite these promising indications, one further issue must be considered in order to develop fully optimized anti-parkinsonian drug therapy, namely the existence of receptor (hetero)dimers/oligomers of G protein-coupled

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receptors, a topic currently the focus of intense debate within the scientific community. Dopamine D_2 receptors (D_2Rs) expressed in the striatum are known to form heteromers with A_{2A} adenosine receptors. Thus, the development of heteromer-specific A_{2A} receptor antagonists represents a promising strategy for the identification of more selective and safer drugs.

1. Introduction

Adenosine receptors (AR) are members of the G protein-coupled receptor superfamily that have long been considered potential targets for the treatment of a variety of diseases, although to date adenosine (Adenocard® or Adenoscan®) is the only commercially available therapeutic drug acting on AR. Adenocard® is used clinically to revert paroxysmal supraventricular tachycardia, while Adenoscan® is also used for cardiac imaging due to its vasodilatory effects mediated by A_{2A} receptors in blood vessels. Recently, the A_{2A}-selective agonist regadenoson (Lexiscan®) was approved for the same indication. Despite the poor selection of available compounds, it is still believed that drugs acting on adenosine receptors will be therapeutically useful. Indeed, five clinical trials are currently underway (phases I to III) to analyze the therapeutic potential of adenosine A_{2A} receptor (A_{2A}R) antagonists in the treatment of Parkinson's disease (PD). Novel adenosine antagonists may thus soon reach the market. The potential of these antagonists has been deduced from considerable investigation of the functional interactions between dopamine and adenosine receptors in the basal ganglia. The use of A_{2A}R antagonists in Parkinson's disease (PD) is based on solid preclinical data showing that adenosinergic neuromodulation antagonizes dopaminergic neurotransmission in aspects relevant to motor control. Adenosine receptor antagonist-based therapy was initially founded on the hypothesis that preventing such antagonism could be useful in situations of dopamine deficit, such as occurs in Parkinson's disease. Notable efforts in medicinal chemistry have sought to develop A_{2A}R antagonists. While the first approaches focused on xanthine derivatives, the current portfolio also includes highly promising non-xanthine drugs.

The use of A_{2A}R antagonists in PD is not exclusively dependent on the outcome of the ongoing clinical trials with structurally distinct molecules. This is due to a shift in emphasis from simply improving the motor symptoms of the patients to developing strategies to prevent disease progression. Given the established efficacy of L-DOPA, and for ethical reasons, the main approach currently used in clinical trials involves the co-administration of A_{2A}R antagonists with L-DOPA. The proposed advantage of this strategy is a reduction in the required dose of L-DOPA, with concomitant reductions in the associated side effects, consisting mainly of dyskinesias and progressive cognitive impairment. Preclinical findings also indicated potential neuroprotective effects of A_{2A}R antagonists, an aspect highly relevant to PD treatment. Thus, in addition to improving motor symptoms when administered in combination with L-DOPA, A_{2A}R antagonists may also exhibit true disease-modifying activity, delaying the progression of disease. Whether all A_{2A}R antagonists being currently assayed in clinical trials are equally effective as co-adjuvants remains to be determined. However, the development of A_{2A}R antagonists for the treatment basal ganglia disorders should focus on optimizing both their effects against acute symptoms and their neuroprotective activity.

An additional and important consideration for the development of A_{2A}R antagonists concerns the novel pharmacological effects derived from G protein-coupled receptor heteromerization. The existence of receptor heteromers has had a strong impact on the field of G protein-coupled receptors, raising important questions as to whether the real therapeutic targets are receptor monomers, homodimers or heteromers. A_{2A}R and dopamine D_2 receptors (D_2R) were among the first G protein-coupled receptor heteromers identified, and

have been detected in both transfected cells and brain striatal tissue (Soriano et al., 2009). Since receptor pharmacology is modified by heteromerization, the screening of given receptors in different heteromeric contexts should be incorporated into future drug discovery programmes. Promising results have been obtained relating to $A_{2A}R$ heteromers (Orrú et al., 2011), which are implicated in Parkinson's and Huntington's diseases (HD), among others. As structurally distinct $A_{2A}R$ antagonists may exert differential effects on distinct $A_{2A}R$-containing heteromers, different $A_{2A}R$ antagonists may be useful for the treatment of specific neurological disorders, depending on the heteromer preferentially targeted by the drug. In this review, we aim to address all these past-, present- and future aspects of the $A_{2A}Rs$ and their antagonists.

2. Normal and abnormal basal ganglia function

PD is a basal ganglia-associated disorder that affects 1-2% of individuals over 60 years of age. The main symptoms of the disease are motor-related, including reduced spontaneous movement, akinesia (lack of movement), bradykinesia (slowness of movements), rigidity (due to increased muscular tone), as well as the characteristic resting tremor. The introduction of the dopamine precursor L-DOPA in the late 1960's, later followed by a number of dopamine agonists, has revolutionized the clinical management of PD (at least within a period of 5-7 years after the first diagnosis, often referred to as the "pharmacological honeymoon"). These therapeutic tools have proven efficient in restoring most of the motor-related deficits characterizing PD. However, even the most effective anti-parkinsonian drugs only provide symptomatic relief. Furthermore, as the disease continues to progress, troublesome side effects generally appear several years after initiation of pharmacological treatment, including motor fluctuations (on-off phenomena) and L-DOPA-induced dyskinesias. In parallel with the appearance of long-term motor disturbances, patients begin to develop non-motor problems, resulting in a marked decline in quality of life. At this stage of disease progression, symptoms like mild cognitive impairment (including dementia in later disease stages), difficulties swallowing and speaking, autonomic dysfunction, gait disturbances and loss of balance (including frequent falls), represent the major causes of disability for PD patients. At present, it seems clear that PD cannot solely be attributed to nigrostriatal dopaminergic deficits. As such the involvement of non-dopaminergic systems, particularly in relation to the appearance of non-motor symptoms, needs to be better defined. In this scenario, current strategies aim to either: (i) slow down the natural progression of PD through disease-modifying therapies, or (ii) better manage the side-effects that appear after long-term treatment with L-DOPA, particularly 'on-off' phenomena and abnormal involuntary movements (*i.e.*: L-DOPA-induced dyskinesia).

The basal ganglia are a group of subcortical nuclei involved in the planning and initiation of movement, and are anatomically and functionally organized into parallel circuits that process different types of information. All basal ganglia-related nuclei are connected through well-established neurochemical circuits. When considering the activity of these ganglia, one should bear in mind that after release, the effects of neurotransmitters ultimately depend on the pre- and post-synaptic localization of a number of receptors, as well as on the relationships established with other inputs received by a given neuron. Briefly, the basal ganglia nuclei are divided into: (i) input nuclei that receive information from the cortex and thalamus, consisting of the *caudate*, *putamen* and *accumbens* nuclei; (ii) output nuclei that send information from the basal ganglia to the thalamus, comprised of the internal division of the *globus pallidus* (GPi) and the *substantia nigra pars reticulata* (SNr); and (iii) intrinsic nuclei, composed of the external division of the globus pallidus (GPe), the subthalamic nucleus (STN) and the *substantia nigra pars compacta* (SNc). In addition to these input, output and intrinsic nuclei, several other nuclei are tightly linked to the basal ganglia system, including the tegmental pedunculopontine nucleus (PPN), the caudal

intralaminar nuclei (CM-Pf complex), and to some extent, the deep cerebellar nuclei that also fulfil motor-related functions.

The current model of basal ganglia function and dysfunction (Albin et al., 1989; DeLong, 1990) was postulated in the late 1980's. This proposes that under tonic dopaminergic conditions, the striatum (known as the caudate-putamen) integrates cortical and thalamic information that then reaches the output nuclei, finally arriving to the cerebral cortex via a thalamic relay. There are two main ways by which striatal information can reach the basal ganglia output nuclei: firstly, via a monosynaptic projection called the "direct pathway" that originates in striatal medium-sized spiny neurons (MSNs) expressing dopamine type 1 receptor (D_1R) and ends in GPi/SNr nuclei; secondly, through a multisynaptic circuit called the "indirect pathway", which begins in striatal MSNs expressing type 2 dopamine receptors (D_2R) that project to the GPe. From the GPe, this latter pathway reaches the output nuclei after establishing a synaptic relay in the STN nucleus. One of the cornerstones of this model is the so-called "dual effect" of dopamine at the striatal level, based on the complementary expression of D_1 and D_2 receptors by striatal MSNs that project through the direct and the indirect pathways, respectively. Accordingly, dopamine exerts D_1-mediated excitation of striatal MSNs projecting to GPi/SNr targets, and D_2-mediated inhibition of striatal MSNs innervating the GPe nucleus. Thus, dopamine exerts a dichotomous effect at the striatal level depending on the type of dopaminergic receptor (D_1R or D_2R) located post-synaptically in striatofugal MSNs. Based on this dichotomous effect of dopamine acting on D_1R and D_2R, activation of the direct pathway is proposed to facilitate movement, whereas activation of the indirect pathway is thought to result in movement inhibition. Although anatomical and functional evidence has challenged this hypothesis (Kawaguchi et al., 1990; Surmeier et al., 2005; Gatev et al., 2006), it has been recently validated through the use of optogenetic techniques (Kravitz et al., 2010).

Following dopaminergic denervation, the aforementioned model predicts that the reduced activation of dopaminergic receptors will decrease the excitation of the D_1R-containing striatofugal neurons of the direct pathway, along with a concomitant disinhibition of D_2R-containing striatal neurons of the indirect pathway. This sequence of events is proposed to provoke exacerbated GABAergic flux from basal ganglia output neurons, ultimately leading to excessive inhibition of thalamocortical projections (Crossmann, 1987; Albin et al., 1989; DeLong, 1990; Obeso et al., 1997). The reverse of this phenomenon is predicted to account for the dyskinetic state associated with continuous L-DOPA treatment. This induces a number of downstream changes in basal ganglia circuits, ultimately resulting in hypoactivity of the basal ganglia output, reducing the inhibition of thalamocortical neurons and leading to excessive activation of cortical motor areas. This view is strongly supported by previous studies (Filion et al., 1991; Lozano et al., 2000). In summary, although the current basal ganglia model served as a good starting point, it is important to stress that basal ganglia organization is far more elaborate than assumed in this model (reviewed in Obeso et al., 2000a; Bar-Gad and Bergman, 2001). Indeed, a number of anatomical, electrophysiological and clinical findings are poorly explained by the current model (Obeso et al., 1997, 2000b; Wichmann and DeLong, 2000; Nambu, 2008). Many of the inherent limitations of this model were imposed by the manner in which it was originally conceived, which was somewhat biased by the preponderance of anatomical and neurochemical data available at the time. The basal ganglia is thus a much more sophisticated and dynamic network than that reflected in the basal ganglia model. In addition to new insights into the function of the basal ganglia at the systems level, recent findings detailing neurotransmission throughout basal ganglia networks should be taken into consideration for a more complete understanding of its organization and activity. This is particularly relevant to the role played by G protein-coupled receptor heteromers in the modulation of striatal neurotransmission, in

normal conditions, under circumstances of dopaminergic depletion and in the dyskinetic state associated with continuous administration of L-DOPA and/or dopamine agonists.

3. The role of A_{2A} receptors (A_{2A}Rs) and A_{2A}R heteromers in the modulation of striatal neurotransmission

The A_{2A}Rs are highly expressed in the basal ganglia and depend on G_s and other interacting proteins for correct transduction of their signals (Burgueño et al., 2003). The striatum is the anatomical region in mammals that most strongly expresses A_{2A}Rs, which are thought to fulfil an important role in the regulation of dopaminergic transmission in the basal ganglia (see Morelli et al., 2009). For instance, A_{2A}Rs co-localize postsynaptically with D_2Rs in GABAergic striatopallidal enkephalinergic MSNs. Stimulation of these postsynaptic A_{2A}R counteracts the inhibitory modulation of NMDA receptor activity mediated by D_2Rs, which includes regulating Ca^{2+} influx, transition to the firing "up" state and modulation of neuronal firing in the "up" state (Azdad et al., 2009; Higley and Sabatini, 2010). This interaction appears to be responsible for most of the locomotor depression and activation provoked by A_{2A}R agonists and antagonists, respectively (Ferré et al., 2008). Adenosine A_{2A}R-mediated activity is usually antagonistic to that mediated by striatal D_2R in MSNs. Indeed, functional antagonism between A_{2A} and D_2 receptors was recently reported in striatal cholinergic interneurons (Tozzi et al., 2011). Overall, adenosine-dopamine antagonism underlies the potential therapeutic benefits of A_{2A}R-selective antagonists in PD. The well known regulation of motor control mediated by A_{2A}Rs under conditions of dopamine depletion is sufficiently solid to merit the clinical trials currently underway (see section 7), which aim to demonstrate the therapeutic efficacy of A_{2A}R antagonists in PD.

The existence of A_{2A}R-containing heteromers was first reported at the beginning of the 21st century (Hillion et al., 2002; Fuxe et al., 2003; Canals et al., 2003; Torvinen et al., 2005). Since then, accumulating evidence suggests that they may be the real targets of therapeutic agents used to combat basal ganglia disorders. Receptor heteromers are the focus of intense research since through heteromerization, receptors become unique functional entities with different properties from those of each of the receptors involved (Ferré et al., 2009). Thus A_{2A}R in A_{2A}R-containing heteromers are different therapeutic targets to A_{2A}Rs that do not form heteromers. The allosteric interaction between different receptors in a given heteromer, *e.g.*, A_{2A}R and D_2R in the A_{2A}R-D_2R heteromer, may modify the pharmacological parameters of A_{2A}R or D_2R-selective radioligands. In fact, as indicated in section 8.1, a given A_{2A}R-selective antagonist may display quite different affinities for the "same" A_{2A}R in different heteromeric contexts. When these allosteric changes are measurable, they may be considered as a biochemical fingerprint of the heteromer. Identification of biochemical fingerprints is one of the few procedures available for the detection of heteromers in native tissues. Using this approach and also by the use of bivalent ligands, A_{2A}R-D_2R heteromers have been detected in brain striatum (Soriano et al., 2009). Moreover, the A_{2A}R-D_2R heteromer has been reported in animal models of PD (data in preparation). Taken together these findings suggest that dopamine-based therapies for PD (such as L-DOPA) may target D_2R-containing heteromers, while the A_{2A}R antagonists proposed for the treatment of PD may target A_{2A}R-containing heteromers (reviewed in Franco, 2009 and Casadó et al., 2009).

When attempting to define the physiological significance of the diverse receptor heteromers, it should be noted that the composition of A_{2A}R-containing presynaptic and postsynaptic heteromers differs. Postsynaptically, A_{2A}R may not only form heteromers with D_2R but also with cannabinoid CB_1 receptors (CB_1R). The apparent need for A_{2A}R activation to mediate the motor-depressant effects of endocannabinoids, which occurs tonically in the presence of significant levels of extracellular adenosine, appears to be dependent on the existence of A_{2A}R-CB_1R heteromers. This "property" of the CB_1R-A_{2A}R heteromer (*i.e.*: the

dependence on $A_{2A}R$ activation for CB_1R engagement, Carriba et al., 2007), predicts that $A_{2A}R$ antagonists produce effects similar to CB_1R antagonists. Indeed, motor depression induced by the bilateral striatal infusion of a CB_1R agonist in rats is completely counteracted by systemic administration of either CB_1R or $A_{2A}R$ antagonists (Carriba et al., 2007). Similarly, genetic inactivation of $A_{2A}R$ in mice significantly decreases the cataleptic and rewarding effects of systemically administered CB_1R agonists in a conditioned place preference paradigm (Andersson et al., 2005; Soria et al., 2006). The existence of postsynaptic CB_1R heteromers provides new indications regarding the potential mechanisms underlying the endocannabinoid-mediated control of striatal function, as these heteromers cannot just be considered as retrograde effectors that inhibit neurotransmitter release.

Given the colocalization of CB_1, A_{2A} and D_2 receptors in striatal neurons and the reported interplay between $A_{2A}R$ and CB_1R, $A_{2A}R$ and D_2R, and CB_1R and D_2R (Hillion et al., 2002; Tebano et al., 2009; Navarro et al., 2009; Ferré et al., 2010), the existence of striatal heterotrimers is a clear possibility. It is important to consider that at the structural level, based on the characterization of the trimer in a heterologous system, a trimer's function is determined by its quaternary structure. Indeed, the structural alterations produced in mutant receptors result in a distinct signalling fingerprint in the trimer. Remarkably, the cross-talk derived from the co-activation of $A_{2A}R$ and D_2R depends on a correct trimer structure, which requires the presence of the 3 receptors. Indeed, this CB_1R-dependent adenosine-dopamine cross-talk may be considered the fingerprint of the heteromer. The existence of $A_{2A}R$-CB_1R-D_2R heteromers in the striatum is based on the detection of the trimer fingerprint in slices from wild type but not CB_1R KO animals (for details, see Carriba et al., 2008 and Navarro et al., 2010). Further studies are required to determine the exact role of $A_{2A}R$-CB_1R-D_2R heteromers in striatal function, particularly in states of dopamine depletion.

Striatal $A_{2A}Rs$ are localized both pre and postsynaptically in glutamatergic terminals, where they heteromerize with A_1 receptors (A_1Rs) and fine-tune glutamate release (Ciruela et al., 2006; Quiroz et al., 2009). These A_1R-$A_{2A}R$ heteromers appear to work as concentration-dependent switches (Ferré et al., 2007), with adenosine acting primarily at A_1Rs at low concentrations but at both A_1Rs and $A_{2A}Rs$ at higher concentrations. Activation of A_1R in the A_1R-$A_{2A}R$ heteromer inhibits glutamate release, while the additional activation of the $A_{2A}R$ produces the opposite effect, acting through a mechanism that seems to involve allosteric modulation of the receptor heteromer and interactions at the G protein level (Ciruela et al., 2006, Ferré et al., 2007). Interestingly, presynaptic $A_{2A}Rs$ are preferentially localized in glutamatergic terminals of corticostriatal afferents to the dynorphinergic MSNs (Quarta et al., 2004; Quiroz et al., 2009). Apart from morphological evidence provided by immunohistochemistry and electron microscopy, patch-clamp experiments in enkephalinergic and dynorphinergic MSNs have functionally demonstrated the segregation of striatal presynaptic $A_{2A}Rs$. Thus, an $A_{2A}R$ agonist and an $A_{2A}R$ antagonist increase and decrease, respectively, the amplitude of excitatory post-synaptic currents induced by the intrastriatal stimulation of glutamatergic afferents measured in enkephalinergic, but not dynorphinergic MSNs. Indeed, a mean-variance analysis indicates a presynaptic locus for the effect mediated by $A_{2A}R$ (Quiroz et al., 2009). These findings suggest a selective $A_{2A}R$-mediated modulation of glutamate release to dynorphinergic MSNs, a scenario which contrasts with the recently proposed role of post-synaptic $A_{2A}Rs$ in the modulation of glutamate release to enkephalinergic MSNs (Lerner et al., 2010).

The data presented in this section indicate that heteromers containing $A_{2A}R$ must be considered when attempting to understand the effects of selective $A_{2A}R$ agonists and antagonists, and when developing compounds to compensate for basal ganglia dysfunction. Two main types of $A_{2A}R$-containing heteromers exist, namely pre- and postsynaptic. The

prototypic presynaptic striatal receptor is the A_1R-$A_{2A}R$ found in glutamatergic terminals. Post-synaptic striatal heteromers containing $A_{2A}R$ in the spines of GABAergic enkephalinergic neurons are formed with D_2R and/or CB_1R. Targeting pre- versus postsynaptic $A_{2A}R$, or vice versa, may represent a useful approach to differentially combat disorders affecting the basal ganglia.

4. OVERVIEW OF IMPORTANT $A_{2A}R$ ANTAGONISTS

Several review articles on adenosine receptor ligands in general (Müller & Jacobson, 2011a; Müller & Jacobson, 2011b; Fredholm et al., 2011) and $A_{2A}R$ antagonists in particular (Müller & Ferré, 2010; Clementina & Giuseppe, 2010; Shah & Hodgson, 2010; Cristalli et al., 2009) have appeared recently. The first adenosine receptor antagonists described in the literature were the plant alkaloids caffeine (**1**) and theophylline (**2**), which are characterized by their core xanthine structure (Figure 1). These compounds are non-selective, relatively weak antagonists with K_i values in the micromolar concentration range for the four human receptor subtypes (see Table 1) and are approximately equally potent at rat A_1, A_{2A} and A_{2B} receptors, though do not act at the rat A_3 receptor subtype.

5. Rationale for the screening of adenosine $A_{2A}R$ antagonists to prevent neurodegeneration in Parkinson's disease

5.1. Neuroprotection and Parkinson's disease - a general outlook

Replenishing the depleted dopamine stores with L-DOPA, its immediate precursor, thus mimicking dopamine-mediated neurotransmission, remains the basis of current PD treatment. Although this replacement therapy offers immediate and effective symptomatic relief, especially in the early stages of the disease, it does not have any influence on the underlying neurodegenerative processes. As a consequence, neuronal cell death progresses over time and is paralleled by a gradual loss of drug efficacy. Thus, steady adaptation is necessary to maintain adequate symptomatic relief, mainly by increasing the doses of dopaminergic drugs, thereby favouring the emergence of side effects, such as dyskinesia and psychiatric disturbances. The discovery of new drugs, or the combined use of known compounds that not only alleviate motor symptoms but that also delay or even halt the loss of dopaminergic neurons, are fundamental issues in generating alternative therapeutic strategies for PD.

The search for neuroprotective therapies in PD has continued for more than a quarter of a century, involving an impressive number of patients thus far (Voss & Ravina, 2008). Some encouraging results have been obtained using rasagiline, a potent MAO-B inhibitor (Olanow et al., 2008; Olanow et al., 2009; Weinreb et al., 2010), which produces disease-modifying effects in PD patients in early treatment stages. Nevertheless, to date no drug demonstrating neuroprotective effects has been unequivocally approved for use in humans. A major drawback in the development of a neuroprotective therapy is the multifactorial nature of PD. The primary causes of the degenerative process underlying the disease remain unclear. Aside from several gene mutations that have been shown to cause familiar PD (Nuytemans et al., 2010), extensive studies using experimental models of PD, as well as large post-mortem studies have identified the presence of numerous cellular and molecular defects. In particular, oxidative stress and excitotoxic mechanisms, mitochondrial and ubiquitin/proteasomal system (UPS) dysfunction, increased MAO (MAO-A and MAO-B) activity, as well as significant neuroinflammatory processes have all been associated with PD (Cookson & Bandmann, 2010). Any of these alterations could, in principle, represent a potential therapeutic target and many have indeed been singularly targeted (Schapira, 2009). It has become evident over the years however, that pathogenetic pathways do not contribute to disease severity in a linear fashion but rather are highly interconnected and act in parallel.

Therefore, processes leading to nigral cell death are likely to represent the reciprocal cumulative interaction of factors that have little effect on their own. Similarly, mechanisms that sustain ongoing cell loss might differ from initial triggers and are likely to change as dopaminergic neurodegeneration progresses. This strongly suggests that drugs with a single target will be unable to compensate for, or correct, complex alterations. To restore altered activities and potentially slow down disease progression, any therapeutic strategy for PD will likely have to: i) act on different pathways simultaneously; and ii) adapt and evolve as the disease progresses. Thus, as the causes of PD are manifold and interconnected, it is clear that a multilateral approach is needed to effectively treat this complex neurodegenerative disease, using a combination of molecules acting on different pathways. Alternatively, different compounds with promiscuous activity could be developed that operate at multiple targets, providing both symptomatic and neuroprotective benefits.

Antagonists of $A_{2A}R$ have proven highly efficient in restoring motor function in animal models of PD and, as described below (see section 7), have produced some encouraging results in clinical trials. Blockade of $A_{2A}R$ may confer neuroprotection against a large spectrum of brain insults in animal models of ischemia, epilepsy, PD, HD and Alzheimer's disease (AD: Gomes et al., 2010). We will review how $A_{2A}R$ antagonists, aside from their well-documented effects on the motor symptoms characteristic of PD, can target several cellular mechanisms implicated in the underlying neurodegenerative process and possibly confer significant neuroprotection in PD.

5.2. Caffeine, $A_{2A}R$ antagonists and PD – when the past meets the present

In past decades, several large convergent follow-up studies involving more than 150,000 subjects, both male and female, have demonstrated an inverse relationship between the consumption of coffee and the risk of developing PD (Costa et al., 2010). In particular, consumption of caffeinated but not decaffeinated coffee was correlated with a reduced risk of developing the disease (Ascherio et al., 2001; Powers et al., 2008; Ross et al., 2000a; Ross et al., 2000b; Saaksjarvi et al., 2008), indicating that caffeine, a non selective A_1/A_{2A} receptor antagonist, is directly implicated in neuroprotection. Indeed, a large body of evidence continues to support the neuroprotective potential of caffeine both *in vitro* and *in vivo*.

In vitro, caffeine (**1**) can protect rat mesencephalic cells from 6-hydroxydopamine (6-OHDA) toxicity, increasing cell viability, reducing the number of apoptotic cells and completely blocking toxin-induced lipid peroxidation (Nobre et al., 2010). Importantly, in these cell cultures caffeine also reduces the number of activated astrocytes and microglia induced by the neurotoxin. Caffeine also protects against 1-methyl-4-phenylpyridinium (MPP^+)-induced mitochondrial complex I inhibition in cerebellar granule neurons (Alvira et al., 2007). Recently, the development of a rapid assay to evaluate neuroprotective agents has confirmed that caffeine can efficiently reduce toxin-induced PD-related abnormalities in SH-SY5Y cells (Yong-Kee et al., 2010). Analogously, caffeine prevented apoptotic cell death and induced phosphorylation of protein kinase B (Akt) in a dose-dependent manner, suggesting that it exerts a protective effect through activation of the PI3K/Akt pathway (Nakaso et al., 2008), a pathway known to be altered in PD (Armentero et al., 2010; Morisette et al., 2010).

In vivo, administration of caffeine in different experimental paradigms (acute vs chronic, pre- vs. post-treatment) protected against nigrostriatal degeneration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice (Chen et al., 2001b; Kalda et al., 2006; Singh et al., 2009; Xu et al., 2002), a well characterized animal model of PD. Recently, caffeine has also been shown to attenuate the loss of dopaminergic neurons in mice treated with a combination of paraquat and maneb (Kachroo et al., 2010), two

pesticides linked to increased risk of developing PD (Costello et al., 2009). Notably, caffeine treatment reduced neuronal cell death and glial activation in lipopolysaccharide (LPS)-treated mice (Brothers et al., 2010), an animal model of dopaminergic degeneration. A recent exhaustive effort to identify genes involved in the molecular mechanisms of caffeine-mediated neuroprotection towards MPTP-induced alterations in mice clearly demonstrated that caffeine can modulate the expression of proteins involved in PD pathogenesis, including UPS related genes, cytochrome oxidase (subunit VIIc) gene and genes involved in the regulation of microglial activation (Singh et al., 2010). Interestingly, recent evidence indicates that caffeine can also counteract alterations to the blood-brain barrier (BBB) known to occur in neurodegenerative disease, including PD (Chen et al., 2010).

Although caffeine can act on both A_1R and $A_{2A}R$, converging evidence demonstrates that blockade of $A_{2A}R$ but not A_1R contributes to its actions in the brain (Fredholm et al., 1999). Indeed, the effects of caffeine *per se* appear to be largely abolished in $A_{2A}R$ knockout mice (El Yacoubi et al., 2000; Huang et al., 2005). Since the initial discovery of the neuroprotective potential of caffeine, numerous $A_{2A}R$ antagonists have been developed, mainly belonging to two chemical classes (see section 4 above; Müller & Ferré, 2010). Xanthine-based antagonists, such as CSC (**6**), DMPX (**4**), and KW-6002 (**5**), or non-xanthine-based $A_{2A}R$ antagonists including SCH-58261 (**10**), all demonstrate a clear potential to reduce toxin-induced neuronal cell death in rodent models of PD (Carta et al., 2009; Chen et al., 2001b; Ikeda et al., 2002; Joghataie et al., 2004; Pierri et al., 2005). Conversely, A_1R antagonists such as DPCPX show no such protective effect (Chen et al., 2001b). Recent evidence has further demonstrated that other methylxanthines that effectively block $A_{2A}R$, such as theophylline (**2**) and paraxanthine (**3**: Xu et al., 2010), can also provide neuroprotection in MPTP mice.

The mechanisms by which $A_{2A}R$ antagonists may attenuate the demise of dopaminergic neurons are still unknown. However, the effects of $A_{2A}R$ antagonists extend beyond those demonstrated in PD models. $A_{2A}R$ antagonists have been shown to decrease excitotoxic lesions in the hippocampus and striatum (Jones et al., 1998a, 1998b; Popoli et al., 2008; Popoli et al., 2002), as well as cerebral and striatal damage associated with ischemia (Monopoli et al., 1998; Phillis, 1995). In $A_{2A}R$ knockout mice, the volume of infarction induced by transient occlusion of the middle cerebral artery is significantly reduced by caffeine (Chen et al., 1999). $A_{2A}R$ antagonists also offer protection in a mouse model of HD (Chen et al., 2007). These converging findings, along with evidence that genetic deletion of $A_{2A}R$ in mice almost completely prevents MPTP-induced nigrostriatal degeneration (Chen et al., 2001a), strongly suggests that caffeine and $A_{2A}R$ antagonists counteract a range of noxious insults affecting different brain regions. Considering the multifactorial and progressive nature of PD, antagonists of $A_{2A}R$ that can act at multiple cellular levels may be good candidates to intervene, slow down or even halt the underlying neurodegenerative process in this disease.

5.3 $A_{2A}R$ antagonists and glutamate toxicity

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and it is involved in many essential brain functions, including synaptic plasticity, memory and learning, and repair. In the basal ganglia, glutamate mediates excitatory transmission at crucial points and is instrumental in the mechanisms that underlie motor symptoms in PD. Under specific conditions glutamate exerts excitotoxic effects due to glutamate-triggered overload of intracellular calcium. Although compensatory mechanisms allow neurons to deal with contained, self-limited increases in calcium influx, they cannot cope with protracted noxious stimuli. Excitotoxicity is considered a crucial component of numerous pathological conditions in the CNS, including PD in which it may contribute to and/or sustain the inherent neurodegeneration (Blandini, 2010). Reducing glutamate overdrive thus

represents an important strategy to favour neuroprotection in PD (Blandini et al., 2001; Piallat et al., 1996).

As mentioned above, $A_{2A}R$ antagonists protect against a wide range of excitotoxic brain insults, from ischemia to HD. About one third of the $A_{2A}R$ immunoreactivity in the brain is observed in cortico-striatal glutamatergic terminals, and under both normal and pathological conditions, adenosine is known to modulate the pre-synaptic release of glutamate via A_1R (inhibition) or $A_{2A}R$ (facilitation). $A_{2A}R$ antagonists can thus reduce glutamate release by enhancing the inhibitory activity of A_1R and therefore may partially exert neuroprotective effects against excitotoxity by modulating (inhibiting) glutamate release from pre-synaptic terminals (Chen et al., 2007; Cunha, 2005).

The SNc is vulnerable to small, non-toxic changes of glutamate levels. In PD, hyperactivity of GABAergic enkephalinergic neurons induced by the release of D_2R-mediated tonic inhibition causes functional alterations of basal ganglia circuits and triggers hyperactivity in the STN. As well as projecting to the GP and SNr, the STN also targets the SNc (Smith et al., 1996). Glutamatergic over-stimulation that may initially enhance the activity of dopaminergic neurons and compensate for their loss, may over time act as a noxious stimulus, further sustaining neuronal death (Shimo & Wichmann, 2009). $A_{2A}R$ antagonists may potentially decrease the glutamate-dependent excitation of GABAergic enkephalinergic neurons through pre- and post-synaptic mechanisms (see above), thereby reducing the STN-dependent glutamate overstimulation of the SNc, further sustaining their proposed anti-excitotoxic effect in PD. Recently, effects on $A_{2A}Rs$ expressed by astrocytes has emerged as an additional mechanism by which $A_{2A}R$ antagonists can modulate glutamate release and consequently, cell death (see below section 5.4.2).

5.4 $A_{2A}R$ antagonists and modulation of neuroinflammatory processes

In the past decade, neuroinflammation has emerged as an important substrate for PD (Hirsch & Hunot, 2009). Indeed, several epidemiological studies have reported an inverse correlation between the chronic consumption of non-steroid anti-inflammatory drugs and the risk of developing PD (Chen et al., 2003). Although inflammatory reactions initially aim to restore physiological tissue function, chronic stimuli, resulting from overt tissue damage and/or protein aggregation may result in a persistent inflammatory response, provoking a feed-forward loop that overwhelms normal mechanisms of control. As such, uncontrolled inflammation may produce toxic factors that amplify underlying disease states. There is significant evidence demonstrating that neuroinflammatory processes participate in the pathophysiology of PD, and gliosis and lymphocyte infiltration have been consistently reported in the SNc of PD patients (McGeer & McGeer, 1998; McGeer et al., 1988; McGeer & McGeer, 2002, 2008; McGeer et al., 2001). Neuroinflammation is also associated with soluble factors - protective or toxic - that are produced and secreted not only by resident cells in the brain (astrocytes and microglia) but also, by peripheral immune cells that can traffic to the brain parenchyma (Koistinaho & Koistinaho, 2005; McGeer & McGeer, 2004; Minghetti, 2005; Perry & Gordon, 1988; Raivich et al., 1999; Tuppo & Arias, 2005). Similarly, the nigrostriatal neurodegeneration caused by all major neurotoxins used to reproduce PD features in animal models (6-OHDA, rotenone, MPTP) is invariably associated with intense glial cell activation (Abbott, 2000; Armentero et al., 2006b; Carta et al., 2009; Sherer et al., 2003), lymphocyte infiltration (Brochard et al., 2009), and the production of soluble inflammatory factors (Armentero et al., 2006a; McGeer et al., 2001). The levels of neuroinflammatory mediators, such as tumor necrosis factor (TNF)-alpha and interleukin-1, are elevated in the brain of PD patients, and in parkinsonian rodents and non-human primates (Barcia et al., 2005; Hirsch et al., 1998; Nagatsu & Sawada, 2005). Importantly, most studies in animal models of PD have demonstrated that neuroprotective strategies that effectively reduce nigrostriatal degeneration are consistently associated with a

reduction in neuroinflammatory processes and vice versa, highlighting the fundamental link between neuroinflammation and neurodegeneration.

Aside from its well documented distribution in basal ganglia nuclei, $A_{2A}R$ is also expressed by cells associated with the neuroinflammatory process, namely astrocytes (Brambilla et al., 2003; Fiebich et al., 1996; Lee et al., 2003; Nishizaki et al., 2002; Wittendorp et al., 2004), microglia (Fiebich et al., 1996; Hasko et al., 2005) and oligodendrocytes (Stevens et al., 2002). Accumulating evidence indicates that $A_{2A}R$ ligands may modulate neuroinflammatory responses associated with neurodegeneration. Caffeine significantly reduces the number of activated hippocampal microglia in aged LPS-treated rats (Brothers et al., 2010) and pre-treatment of MPTP-mice with $A_{2A}R$ antagonists is associated with weaker glial activation in both the SNc and striatum (Carta et al., 2009; Ikeda et al., 2002; Pierri et al., 2005; Pinna et al., 2010; Yu et al., 2008)

Recently, studies using a conditional mouse knockout of forebrain $A_{2A}R$ (fb-$A_{2A}R$ KO mice: Yu et al., 2008) have emphasised the importance of glial $A_{2A}R$ expression in nigrostriatal degeneration. These mice do not suffer the well-characterized motor effects produced by the $A_{2A}R$ antagonist KW-6002. Moreover, fb-$A_{2A}R$ KO mice show the same susceptibility towards elevated acute MPTP neurotoxicity as their wild type littermates (Yu et al., 2008), and both nigrostriatal degeneration and glial activation can be reduced by additional $A_{2A}R$ blockade with KW-6002 (**5**). Interestingly, the dopaminergic striatal terminals and nigral neurons of fb-$A_{2A}R$ KO mice exposed to weak chronic MPTP intoxication suffer little or no damage, though reduced glial activation is evident (Carta et al., 2009). Importantly, the antagonist doses used to achieve neuroprotection were several times lower than those required to induce motor effects. Hence, the cellular and molecular mechanisms involved in $A_{2A}R$-mediated neuroprotection are likely to be distinct from those required mediating motor effects, and likely occur through glial cells.

5.4.1 $A_{2A}R$ antagonists and microglial cells—Microglia are among the main resident immune cells in the brain. Under physiological conditions, microglial cells exhibit what has been defined as a “surveying” phenotype, characterized by a small soma and long processes. These cells constantly and randomly scan their surrounding microenvironment, rapidly reacting to any local disturbances by producing soluble factors that influence nearby neurons and astrocytes. It is now well established that microglia continuously engage in minor transient and self-limiting repairs that rapidly resolve such situations and generally go unnoticed (Hanisch & Kettenmann, 2007). Within minutes of brain damage these cells send microglial processes to the site of injury, guided by specific local chemoattractants released from affected tissue (such as ATP or ADP), a response that is dependent on stimulation of the purine receptor $P2Y_{12}$ ($P2Y_{12}R$) expressed by microglia (Davalos et al., 2005). However, this situation changes under conditions of prolonged chronic brain damage, such as that observed in neurodegenerative diseases like PD. Following chronic activation, microglia typically assume an amoeboid morphology with highly retracted processes (Block et al., 2007; Hanisch & Kettenmann, 2007; Kreutzberg, 1996; Stence et al., 2001). While this retraction is also driven by ATP, it is characterized by the repulsion from ATP, and correlates with the down-regulation of $P2Y_{12}R$ and up-regulation of $A_{2A}R$ by the activated cells (Haynes et al., 2006; Moller et al., 2000; Orr et al., 2009).

The use of transgenic mice that express the enhanced green fluorescent protein (eGFP) under the control of $A_{2A}R$ promoter has further confirmed that inflammatory stimuli, such as intracerebral LPS injection, can induce strong up-regulation of $A_{2A}R$ in microglial cells. Furthermore, inducers of brain inflammation such as TNF-alpha and beta-amyloid can down- and up-regulate of $P2Y_{12}R$ and $A_{2A}R$ expression, respectively (Orr et al., 2009), a phenomenon that has also been described in human macrophages and brain tissues from AD

patients (Angulo et al., 2003). Interestingly, when $A_{2A}R$ is activated in induced microglia using selective agonists (5'-N-ethylcarboxamide adenosine, NECA, and its C2-substituted derivative, CGS-21680), withdrawal of the cellular processes is stimulated and the characteristic highly activated amoeboid phenotype of microglial cells is adopted (Fredholm et al., 2001; Ongini & Fredholm, 1996). Conversely, when LPS-injected Cx3Crl-eGFP transgenic mice that specifically express the eGFP protein in microglia (Davalos et al., 2005; Haynes et al., 2006; Nimmerjahn et al., 2005) are treated with the $A_{2A}R$ antagonist SCH-58261(**10**), the repulsion of activated microglial processes from ATP at the inflamed site is attenuated, and the reversion of microglial cells from a highly activated amoeboid phenotype towards a less activated phenotype with processes is impaired (Orr et al., 2009). These results are in agreement with the observation that reduced nigrostriatal degeneration in MPTP mice treated with the $A_{2A}R$ antagonist KW-6002 (**5**) is accompanied by a reduction in neuroinflammation, characterized by a specific decrease in the number of large, activated amoeboid microglial cells (Yu et al., 2008). These data clearly indicate that $A_{2A}R$ plays a fundamental role in the stepwise activation of microglia. $A_{2A}R$ antagonists may help limit the development of a fully activated, amoeboid, noxious microglial phenotype, thereby breaking a vicious circle in which neurodegeneration and neuroinflammation sustain each other. Interestingly, it can be inferred that treatment with $A_{2A}R$ antagonists may be "useful" at later stages of the neuroinflammatory process when advanced microglial activation is evident.

The functional plasticity of microglial cells is not only evident through changes in morphology and the expression of cell surface receptors, but also through modified production and release of soluble factors. Microglia represent a source of neurotoxic factors that can further drive neuronal damage, and the extreme vulnerability of the SNc to oxidative stress is likely correlated with a greater microglial density as compared with in other brain regions (Lawson et al., 1990). Alterations in the levels of soluble modulators such as nitric oxide (Knott et al., 2000), IL-1beta, IL-6, IFN-gamma, and TNF-alpha have been detected in the SNc of PD patients (Marchetti & Abbracchio, 2005; McGeer & McGeer, 2008; Tansey & Goldberg, 2010; Whitton, 2007). Moreover, $A_{2A}R$ stimulation can enhance nitric oxide release by activated microglia, while blockade of the receptor with the antagonist ZM-241385 (**9**) significantly suppresses the release of this noxious molecule (Saura et al., 2005). Thus, attenuation of nitric oxide production could contribute in part to the neuroprotective effect afforded by $A_{2A}R$ antagonists. Similarly, ZM-241385 (**9**) reduced IL-1beta activation in quinolic acid-treated rats (Stone & Behan, 2007), SCH-58261 (**10**) prevented cyclooxygenase (COX)-2 expression by striatal microglial cells (Chen et al., 2007), and blockade of $A_{2A}R$ with caffeine (**1**) diminished TNF-alpha release from LPS-activated monocytes (Chavez-Valdez et al., 2009).

Activation of the mitogen-activated protein kinase (MAPK) p38 signalling cascade causes cytokine release and has been implicated in PD pathogenesis. Inhibitors of the MAPK p38 pathway thus represent potential therapeutic agents for the treatment of neurodegenerative diseases (Yasuda et al., 2010). Recently, repeated administration of the selective $A_{2A}R$ antagonist SCH-58261 (**10**) was shown to reduce MAPK p38 activation in microglial cells (Melani et al., 2006). These data suggest that blocking $A_{2A}R$ may impede neurodegeneration by modulating the release of noxious factors by activated microglia.

5.4.2. Effects of $A_{2A}R$ antagonists on astrocytes—Astrocytes are strategically localized in close contact with neuronal structures in all regions of the brain. It has now become evident that astrocytes do not solely endow a structural support to neurons but also play a fundamental role in maintaining their environment. Astrocytes are considered an essential part of a neuronal-glial-vascular unit, strictly controlling local blood flow,

supplying neurons with energy substrates and representing a fundamental homeostatic element in the brain (Magistretti, 2006; Mulligan & MacVicar, 2004; Zonta et al., 2003).

Like microglia, astrocytes react rapidly to CNS injury and their activation has been reported consistently in PD patients (McGeer & McGeer, 2008) and in toxin-induced animal models of the disease (Armentero et al., 2006a; Carta et al., 2009). Nevertheless, the precise neuroprotective and/or neurotoxic influence of activated astrocytes in PD remains poorly understood, and appears to rely on the molecules released into and taken up from the extracellular space. Beneficial effects of astrocytes include the support and sustenance of correct neural function, derived from the initial release of neurotrophic factors, including glial-derived neurotrophic factor (GDNF) and nerve growth factor (NGF), as well as of antioxidant molecules like glutathione. However, protracted astrogliosis may be detrimental due to the secretion of neurotoxic substances that hinder functional recovery or induce damage. Astrocytes normally support axonal growth (Privat, 2003), although prolonged activation can lead to formation of a glial scar at the site of lesion and inhibit axon regeneration (Rolls et al., 2009). Significant and persistent phenotypic changes, such as hypertrophy and up-regulation of glial fibrillary acidic protein (GFAP), have been observed in activated astrocytes. Inflammation *per se* has been shown to enhance the density of A_{2A}Rs on astrocytes, leading to an increase in proliferation and activation of the cells. Stimulation of A_{2A}R can induce the secretory activity of the cells (DeLeo & Yezierski, 2001; Fiebich et al., 1996) and further enhance their proliferation and activation (Brambilla et al., 2003; Bura et al., 2008; Minghetti et al., 2007). Conversely, the A_{2A}R antagonists DPMX (**4**), SCH-58261 (**10**) and KW-6002 (**5**), have opposite effects (Brambilla et al., 2003), and A_{2A}R blockade downregulates GFAP immunoreactivity in primary astrocytes and rodent models of neurodegeneration (Brambilla et al., 2003; Ke et al., 2009; Minghetti et al., 2007). Consistent with these findings, A_{2A}R knockout mice display limited astroglial growth (Bura et al., 2008).

The blockade of A_{2A}R in astrocytes appears to reduce noxious astrogliosis and neurodegeneration. Indeed, astrocytes fulfil an important function by buffering neurotransmitter activity, which can be modulated through A_{2A}R. There is also evidence that the A_{2A}R modulates glutamate release and uptake from astrocytes (Cunha, 2005) and is involved in intracellular calcium release in astrocytes (Doengi et al., 2008). Chronic neuroinflammation is associated with inhibition of glutamate uptake and enhanced release of glutamate by astrocytes (Bezzi et al., 2001), a phenomenon further amplified by activated microglia (Rothwell et al., 1997). Indeed, A_{2A}R agonists can enhance glutamate efflux in cultured astrocytes while receptor blockade significantly reduces the levels of extracellular glutamate (Chen & Pedata, 2008; Li et al., 2001; Nishizaki et al., 2002). Thus, A_{2A}R antagonists could potentially modulate astrogliosis to reduce inflammatory burden in PD and alleviate the excitotoxicity associated with the incorrect handling of extracellular glutamate by activated astrocytes.

5.4.3. Effect of A_{2A}R antagonists in the blood-brain-barrier and peripheral immune system—The blood-brain barrier (BBB) is a fundamental separation between the CNS and systemic circulation that regulates and protects the brain microenvironment. BBB integrity favours proper CNS functioning and its alteration leads to changes in neuronal behaviour and survival (Zlokovic, 2008). The stability of the BBB depends mainly on the presence of the tight junctions that form between adjacent endothelial cells, which are tightly regulated at both the protein level and by multiple cell signalling pathways (Abbott & Revest, 1991; Ishizaki et al., 2003; Stelzner et al., 1989). BBB disruption has been reported to contribute to PD progression in patients and various animal models of the disease (Kortekaas et al., 2005; Carvey et al., 2005; Zhao et al., 2007; Stolp & Dziegielewska, 2009; Weiss et al., 2009). In particular, endothelial alterations in the SNc of PD patients have been

detected (Faucheux et al., 1999). In addition, disruption of BBB permeability *per se* can induce dopaminergic neurodegeneration (Rite et al., 2007), while both neuroinflammation and oxidative stress compromise BBB permeability.

In MPTP-treated mice, chronic ingestion of caffeine (**1**) protects against toxin-induced BBB dysfunction. In these mice, caffeine significantly reduced the disease-related Evan's blue and albumin leakage in the striatum, as well as the diminished expression of tight junction protein, indicative of BBB dysfunction (Chen et al., 2008). The $A_{2A}R$ is also strongly expressed by brain endothelial cells (Phillis, 1989; Schaddelee et al., 2003) in which caffeine likely modulates cAMP levels and affects the release of calcium from intracellular stores (Chen et al., 2010). Interestingly, by acting through $A_{2A}R$ caffeine can also protect the BBB by modulating lipid and/or cholesterol metabolism (Reiss et al., 2004). Activated glial cells release pro-inflammatory and neurotoxic factors, including TNF-alpha, reactive oxygen species and interleukins, all of which alter the BBB (Abbott, 2000). Therefore, caffeine may also help maintain BBB integrity by modulating other cell types including astrocytes, microglia and neurons (see above).

If the BBB is disrupted, peripheral immune cells can cross into the brain parenchyma, further enhancing neuroinflammatory processes, thereby creating a noxious feed-forward loop. In chimeric mice in which $A_{2A}R$ can be selectively inactivated in bone marrow-derived cells (Dai et al., 2010), important cellular regulators of inflammation in the CNS (Yu et al., 2004) attenuate neurological deficits and reduce cell apoptosis in mice following traumatic brain injury. This protective effect is probably linked to the inhibition of glutamate and inflammatory cytokine release. Recent work has indicated that peripheral cells, including lymphocytes, also express detectable levels of $A_{2A}R$, the expression of which is augmented in PD patients (Varani et al., 2010). Thus, modulation of BBB permeability and the circulation of immune cells from the periphery to the brain parenchyma following modulation of $A_{2A}Rs$ may be an important therapeutic intervention in PD.

5.5 $A_{2A}R$ antagonists and MAO inhibition

MAOs (MAO-A and MAO-B) constitute the major catabolic pathway for dopamine in the striatum and they have been considered an important target for PD treatment. MAO-B is predominantly expressed by glial cells (Levitt et al., 1982), and its activity increases both with age and glial activation (Fowler et al., 1997; Youdim & Bakhle, 2006). Inhibition of MAO-B may slow the depletion of dopamine stores and elevate the levels of both endogenous dopamine and dopamine produced from exogenously administered L-DOPA (Finberg et al., 1998). Furthermore, oxidation of dopamine by MAO-B leads to the generation of H_2O_2, which can readily react with free iron (II) ions, exacerbating neurodegeneration. Inhibitors of MAO-B may confer neuroprotection by decreasing potentially hazardous by-products in the brain (Sagi et al., 2007). Accordingly, rasagiline, a potent selective inhibitor of MAO-B, exhibits significant neuroprotective activity in various animal models of PD (Blandini et al., 2004; Sagi et al., 2007) and has demonstrated some benefits in slowing disease progression in a phase III delayed-start clinical study (Olanow et al., 2008; Olanow et al., 2009; Weinreb et al., 2010). Reversible inhibition of MAO-B activity has been reported for several $A_{2A}R$ antagonists (Castagnoli et al., 2003; Chen et al., 2001; Petzer et al., 2009; Petzer et al., 2003; Pretorius et al., 2008; Vlok et al., 2006), including very high concentrations of KW 6002 (**5**: K_i = 28 μM) and CSC (**6**, K_i = 80.6 nM), two antagonists that significantly reduce nigrostriatal degeneration in the MPTP mouse model. These results indicate that the neuroprotective properties of CSC may partly involve MAO-B inhibition in the brain, in synergy with $A_{2A}R$ antagonism.

5.6 Future directions

The past two decades have provided substantial information regarding the neuroprotective potential of caffeine and $A_{2A}R$ antagonists. The selective blockade of $A_{2A}R$ has been shown to benefit many brain disorders caused by a wide spectrum of insults, though the mechanisms underlying their neuroprotective potential in PD remain to be established. As indicated above, accumulated data suggests that this neuroprotective influence may differ from, and/or be complementary to the well-characterized motor stimulatory effects of these compounds. Indeed, the mechanisms underlying the neuroprotective role of $A_{2A}R$ against nigrostriatal degeneration appear to be complex, requiring further study. $A_{2A}R$ may afford neuroprotection by modulating glutamate release and uptake, thereby reducing the excitotoxic burden, as well as by diminishing the production of toxic metabolites through the inhibition of MAO-B. $A_{2A}R$ antagonists may also alter the behaviour of the BBB and the transit of peripheral immune cells to the damaged brain parenchyma. Importantly, $A_{2A}R$ antagonists may affect glial activation and limit, or even revert, the development of fully activated noxious microglia and astrocytes, thereby influencing neurodegeneration and neuroinflammation. The existence of heteromerization and crosstalk between $A_{2A}R$ and other G protein-coupled receptors has also been demonstrated, whereby blockade of $A_{2A}R$ can modulate the activity of companion receptors and further enhance their positive effects against dopaminergic cell loss.

Although it is well accepted in animal models of PD that $A_{2A}R$ expression increases in neurons (Rebola et al., 2005) and glia (Yu et al., 2008) under noxious conditions (Cunha, 2005), better definition of the pharmacological properties, localization and disease-related modifications of $A_{2A}R$ in the human brain is required, both in terms of anatomical expression and heteromerization. It is evident that the phase of the disease at which an antagonist is administered is a critical factor in terms of the magnitude of the effect and the final outcome. Hence, the development of new compounds acting on receptor heteromers containing $A_{2A}R$ represents an innovative and potentially beneficial therapeutic strategy for the treatment of PD.

6. Rationale behind the screening of adenosine $A_{2A}R$ antagonists to improve motor symptoms in animal models and Parkinson's disease

The use of $A_{2A}R$ agonists has been associated with side effects, with 184 out of 334 patients (20 out of 170 in the placebo group) reporting adverse effects in the clinical trial NCT00863707 of regadenoson administered by intravenous bolus injection (0.4 mg/5 ml: available at http://clinicaltrials.gov), including headache, nausea, chest discomfort, dyspnoea or dizziness. Nevertheless, $A_{2A}R$ agonists like regadenoson are safe and well tolerated when applied as pharmacological stress agents for myocardial perfusion imaging, even after heart transplant (Cavalcante et al., 2011). By contrast, $A_{2A}R$ antagonists are generally quite safe, as demonstrated in clinical trials with KW-6002 and preladenant (see below) in which treated patients do not display stable alterations in blood pressure or signs of irritability or anxiety. Therefore, the phenotype described for $A_{2A}R$ KO mice does not occur in humans treated with $A_{2A}R$ antagonists. Thus, while $A_{2A}R$ agonists may be suitable for use in acute controlled (myocardial perfusion imaging) or topical interventions, antagonists appear to be safe even when administered by chronic oral treatment. In reviewing the preclinical and clinical data from the use of $A_{2A}R$ antagonists, and the acute and chronic effects of different $A_{2A}R$ antagonists in rodent and non-human primate models of PD, their potential as non-dopaminergic medication in the therapy of parkinsonian patients can be assessed.

6.1. Reduction of motor symptoms by A_{2A}R antagonists

The essential role of A_{2A}R in modulating motor activity has been established in behavioural studies employing selective ligands. Indeed, administration of the A_{2A}R agonist CGS-21680 inhibits motor behaviour (Janusz & Berman, 1992; Barraco et al., 1993; Karcz-Kubicha et al., 2003), whereas the A_{2A}R antagonist SCH-58261 stimulates motor activity (**10**: Svenningsson et al., 1997; Halldner et al., 2000; Lindskog et al., 2002). Consequently, beneficial effects of A_{2A}R blockade on motor deficits have been demonstrated in a number of animal models of PD, including reversion of catalepsy induced by haloperidol or of hypomotility by reserpine and modulation of turning behaviour in unilateral 6-OHDA-lesioned rodents, as well as attenuation of motor impairment in MPTP-treated non-human primates (Xu et al., 2005; Simola et al., 2008).

Several A_{2A}R antagonists effectively counteract catalepsy in rodents, reducing its duration and severity, thereby improving parkinsonian motor impairment (Kanda et al., 1994; Shiozaki et al., 1999; Villanueva-Toledo, 2003; Pinna et al., 2005; Stasi et al., 2006; Gillespie et al., 2009; Hodgson et al., 2009). Furthermore, the co-administration of L-DOPA with A_{2A}R antagonists such as KW-6002 (**5**), KF 17837 or ST-1535 (**14**) strengthens the anticataleptic effect of the former, indicating the existence of a synergistic interaction between L-DOPA and A_{2A}R antagonists (Kanda et al., 1994; Shiozaki et al., 1999; Stasi et al., 2006). As in the catalepsy model, acute administration of several A_{2A}R antagonists to unilateral 6-OHDA-lesioned rats significantly potentiates turning behaviour induced by L-DOPA or apomorphine, and by D_1R or D_2R agonists (Vellucci et al., 1993; Pollack & Fink, 1996; Pinna et al., 1996, 2005, 2010; Fenu et al., 1997; Koga et al., 2000; Rose et al., 2007; Tronci et al., 2007; Hodgson et al., 2009). In addition to turning behaviour, finer features of PD symptoms resulting from neuron degeneration have been assessed in unilateral 6-OHDA-lesioned rats, including forelimb akinesia, gait impairment and sensory-motor integration deficits. These deficits are considered similar to PD-associated symptoms in humans and have been measured in specific tests, such as initiation of stepping time, adjusting step counting and vibrissae-elicited forelimb placing tests (Olsson et al., 1995; Schallert et al., 2000). Like the L-DOPA effect, A_{2A}R blockade reversed the impairment observed in these tests, suggesting that in PD patients not afflicted by L-DOPA related side-effects, A_{2A}R antagonists may ameliorate diverse parkinsonian symptoms, even when administered as a monotherapy (Pinna et al., 2007, 2010).

Other studies have indicated that A_{2A}R antagonists exert beneficial effects in rat models of parkinsonian rigidity and resting tremor, which can be as disabling as bradykinesia and akinesia. The clinical muscular rigidity, characterized by increased resistance to passive movement, can be mimicked in rodents with adequate doses of haloperidol or reserpine, inducing muscle rigidity with mechanographic and electromyographic features similar to those observed in PD patients (Lorenc-Koci et al., 1996). These effects are reversed by A_{2A}R blockade with SCH-58261 (**10**), (Wardas et al., 2001; Wardas, 2003). Moreover, combined administration of SCH-58261 with L-DOPA, which alone has no effect on haloperidol- or reserpine-induced muscle rigidity, induced pronounced synergistic effects and marked alleviation of the symptoms (Wardas et al., 2001; Wardas, 2003). These beneficial effects on parkinsonian-like muscular rigidity of A_{2A}R antagonists are likely mediated by the facilitation of postsynaptic dopamine transmission (Wardas et al., 2001; Wardas, 2003).

The antitremorigenic effects of drugs can be evaluated in rodents by measuring their ability to reverse tremulous jaw movements induced by several pharmacological agents, including the cholinesterase inhibitor tacrine, the muscarinic agonist pilocarpine, haloperidol and the neurotoxin 6-OHDA (Salamone et al., 1998). Acute administration of A_{2A}R antagonists significantly reversed jaw tremor stimulated by tacrine, haloperidol or pimozide in rats,

suggesting these compounds may be useful to specifically combat this parkinsonian symptom (Correa et al., 2004; Simola et al., 2004, 2006; Tronci et al., 2007; Salamone et al., 2008; Pinna et al., 2010). Moreover, infusion of the A_{2A}R antagonist SCH-BT2 in different regions of striatum provided evidence that the ventrolateral but not the dorsolateral striatum was critical to completely reverse tacrine-induced tremulous jaw movements (Simola et al., 2004). On the basis of the critical role of increases in striatal acetylcholine in the genesis of tremulous jaw movements (Salamone et al., 1998), modulation of cholinergic transmission by A_{2A}R antagonists may underlie the anti-tremorigenic effects of these drugs. Indeed, in line with results obtained in rodents, acute administration of A_{2A}R antagonists increased locomotor activity in a dose-dependent manner and they reversed motor disabilities in non-human primates previously rendered parkinsonian with the dopaminergic neurotoxin MPTP (Kanda et al., 1998, 2000; Grondin et al., 1999; Rose et al., 2006; Hodgson et al., 2010). Moreover, A_{2A}R antagonists act synergistically with L-DOPA to restore motor deficits, as well as with dopamine D_1R and D_2R agonists, in MPTP-treated non-human primates (Kanda et al., 2000; Rose et al., 2006; Hodgson et al., 2010).

To summarize, findings obtained in animal models of PD strongly indicate that acute administration of A_{2A}R antagonists not only ameliorate motor impairment but also effectively counteract parkinsonian-like muscle rigidity and resting tremor. Notably, these latter effects are of particular interest for clinical application of A_{2A}R antagonists since in parkinsonian patients, muscle rigidity and resting tremor are often resistant to commonly prescribed antiparkinsonian drugs. Moreover, synergistic interactions between L-DOPA and A_{2A}R antagonists have been described in different experimental models, suggesting that they may be co-administered to potentiate the motor stimulant effects.

6.2. Reduction of dyskinesia by A_{2A}R antagonists

As seen for acute administration, chronic administration of A_{2A}R antagonists effectively improves motor deficits in animal models of PD and does not produce tolerance to the motor stimulant effects. By contrast, the non-specific adenosine antagonist caffeine loses its motor stimulant effect after repeated administration (Fredholm et al., 1999; Halldner et al., 2000). In unilateral 6-OHDA-lesioned rats, the potentiation of the intensity of L-DOPA-induced turning behaviour elicited by acute SCH-58261 (**10**) administration can be observed even after two weeks of repeated daily treatment with this A_{2A}R antagonist (Pinna et al., 2001). Similar results were reported following the combined administration of KF-17837 or KW-6002 (**5**) and apomorphine, which specifically increased the duration rather than the intensity of turning behaviour (Koga et al., 2000). In MPTP-treated common marmosets, chronic treatment with KW-6002 (**5**) also attenuated parkinsonian motor disability with no sign of tolerance (Kanda et al., 1998). Moreover, like the increased duration of apomorphine-induced turning behaviour produced by acute treatment with A_{2A}R antagonists, co-administration of KW-6002 (**5**) and L-DOPA prevents the shortening of turning behaviour induced by chronic L-DOPA, reflecting a possible benefit of A_{2A}R blockade on the L-DOPA-induced “wearing off” phenomenon observed in PD patients (Koga et al., 2000; Oh & Chase, 2002; Bibbiani et al., 2003). However, this effect on “wearing off” was not confirmed with the A_{2A}R antagonist CSC (**6**), which appears to reverse but not prevent the decrease in motor response duration induced by repeated L-DOPA administration (Bové et al., 2002).

Interesting results have been obtained concerning the modulation of dyskinesia by A_{2A}R blockade when comparing the sensitization of turning behaviour and/or the development of abnormal involuntary movements (AIMs) elicited by long-term treatment of a full dose of L-DOPA (rodent models of dyskinesia) with an equipotent combination of a lower dose of L-DOPA plus a A_{2A}R antagonist (Pinna et al., 2001; Tronci et al., 2007; Hodgson et al., 2009). While treatment with L-DOPA (high dose) and L-DOPA (lower dose) plus SCH-58261 (**10**),

preladenant (**12**) or ST-1535 (**14**) produced a comparable degree of turns upon the first administration, sensitization of turning behaviour and/or AIMs was observed in response to chronic L-DOPA alone but not when administered with an $A_{2A}R$ antagonist (Pinna et al., 2001; Tronci et al., 2007; Hodgson et al., 2009).

The stable response observed after long-term administration of L-DOPA with an $A_{2A}R$ antagonist suggests that the association between the two drugs represents a treatment with lower dyskinetic potential. Interestingly, this hypothesis was supported by studies showing that genetic deletion of the $A_{2A}R$ prevents the sensitization of turning behaviour and AIMs stimulated by L-DOPA in 6-OHDA-lesioned mice (Fredduzzi et al., 2002; Xiao et al., 2006). Indeed, 6-OHDA-lesioned rats treated with KW-6002 (**5**) did not develop any AIMs (Lundblad et al., 2003), while motor disabilities assessed with a rotarod test improved. However, KW-6002 (**5**) did not prevent the severity of AIMs induced by L-DOPA in this study when the two drugs were chronically co-administered and L-DOPA given at a full dose. Hence, co-treatment with an $A_{2A}R$ antagonist and L-DOPA did not prevent the development of AIMs if L-DOPA was given at a full dose to severely dopamine-denervated rats (Lundblad et al., 2003).

The findings from rodent models of dyskinesia have been confirmed and expanded in MPTP-treated marmoset and cynomolgus monkeys. Firstly, in MPTP-treated non-human primates previously rendered dyskinetic by chronic L-DOPA, $A_{2A}R$ antagonists induced no dyskinesia *per se* (Kanda et al., 1998; Grondin et al., 1999; Hodgson et al., 2010). Furthermore, no sign of dyskinesia was observed in parkinsonian cynomolgus monkeys chronically treated with apomorphine and KW-6002 (**5**: Bibbiani et al., 2003). In fact, in dyskinetic MPTP-treated common marmosets the relief of motor impairment produced by an optimal dose of L-DOPA, which presented a high dyskinetic potential, was similar to that of the combination of KW-6002 (**5**) or preladenant (**12**) plus a suboptimal dose of L-DOPA, which was associated with poor induction of dyskinesia (Kanda et al., 2000; Hodgson et al., 2010). Interestingly, no exacerbation of dyskinetic movements was observed after brief administration of KW-6002 (**5**) plus L-DOPA to dyskinetic MPTP-treated marmosets, further supporting the potential of $A_{2A}R$ antagonists in palliating dyskinesia (Kanda et al., 2000).

Taken together, data from preclinical studies indicates that chronic $A_{2A}R$ antagonist administration has beneficial effects on PD motor impairment and on motor complications produced by long-term L-DOPA treatment. These effects are of considerable interest given that motor complications represent an intrinsic limitation of L-DOPA therapy that is often insensitive to pharmacological manipulation. Moreover, since little tolerance to the motor effects of $A_{2A}R$ antagonists is evident, these drugs may be used successfully over long periods, rendering them particularly suitable for a prolonged pharmacological treatment such as that required in PD.

7. Effects of $A_{2A}R$ antagonists in parkinsonian patients: results from clinical trials

The development of new highly selective adenosine $A_{2A}R$ antagonists, and their encouraging antiparkinsonian responses in animal models of PD, has provided a rationale for clinical trials to evaluate the therapeutic potential and the safety of these agents in PD patients.

7.1. KW-6002 (istradefylline)

Although early clinical trials were prompted by several Pharmaceutical Companies, the vast majority of data available on the effectiveness of $A_{2A}R$ antagonists in PD patients has been collected using the xanthine derived compound istradefylline (also named KW-6002: **5**) produced by Kyowa Hakko Kyogo (Jenner, 2005).

In early phase IIa clinical trials with KW-6002 (at doses between 20-80 mg/day), patients with moderate-to-severe PD and overt motor complications were evaluated using the motor Unified PD Rating Scale (UPDRS motor: Bara-Jimenez et al., 2003; Hauser et al., 2003). These studies demonstrated that when administered alone, KW-6002 (40 or 80 mg/day) has no effect on either motor impairment or dyskinesia in PD patients (Bara-Jimenez et al., 2003), whereas in conjunction with a low dose of L-DOPA (which alone has no antiparkinsonian effect), KW-6002 (80 mg/day) significantly improves motor impairment to a similar extent to an optimal dose of L-DOPA, while eliciting weaker dyskinetic effects. Indeed, co-administration led to a beneficial effect on all cardinal symptoms in PD patients, particularly on resting tremor (Bara-Jimenez et al., 2003). When co-administered with a standard amount of L-DOPA, all doses of KW-6002 prolonged the half-life of the optimal L-DOPA dose, indicating that KW-6002 reduced the time spent in the L-DOPA off state (OFF Time: Bara-Jimenez et al., 2003; Hauser et al., 2003). Moreover, while the severity of dyskinesia remained unchanged, the ON Time with dyskinesia increased following KW-6002 administration (Bara-Jimenez et al., 2003; Hauser et al., 2003).

In two large phase IIb trials (US-005 and US-006) and a phase III (US-013) trial carried out on advanced PD patients suffering “wearing-off” (with or without dyskinesia), treatment with L-DOPA alone or in combination with other PD medications confirmed that KW-6002 (at all doses between 20-60 mg/day) produced a significant decrease in L-DOPA OFF Time in PD patients (LeWitt et al., 2008; Stacy et al., 2008; Hauser et al., 2008). Importantly, these studies included an essential measure to differentiate between “troublesome” and “non-troublesome” dyskinesia during L-DOPA ON Time, demonstrating that KW-6002 significantly increased L-DOPA ON Time with non-troublesome dyskinesia, whereas L-DOPA ON Time with troublesome dyskinesias remained unchanged (LeWitt et al., 2008; Stacy et al., 2008; Hauser et al., 2008). Interestingly, a long phase III clinical study (US-007) of PD patients who had previously been involved in other studies (US-001, US-005 and US-006) showed that the efficacy of KW-6002 in reducing the OFF Time at doses of between 20 and 60 mg/day was maintained in patients who were already taking the drug at the onset of the study, providing evidence of consistent and sustained effects (Factor et al., 2010). Similar efficacy of KW-6002 was confirmed in a Japanese phase III study (6002-0608) in which the OFF Time was not only significantly reduced at doses of 20 and 40 mg/day but also, UPDRS motor scores were seen to improve (Mizuno et al., 2010). Notably, KW-6002 (40 mg/day) was recently assessed as a monotherapy in early PD patients (US-051 trial: Fernandez et al., 2010); albeit the improvement was not statistically significant across groups, UPDRS motor scores were better at all time points (and significantly better at week two) (Fernandez et al., 2010). The inability of KW-6002 monotherapy to reverse parkinsonian disability in humans contrasts with findings in animal models of PD (Kanda et al., 1998, 2000; Grondin et al., 1999; Shiozaki et al., 1999; Pinna et al., 2007). This discrepancy may be explained by the low doses used in clinical trials. Higher doses of KW-6002 may be required when administered alone to elicit beneficial effects upon parkinsonian symptoms.

Some safety concerns were raised following observations in $A_{2A}R$ knockout mice of increased blood pressure, as well as irritability and anxiety. However, in all clinical trials KW-6002 has appeared to be well tolerated and safe. No notable differences were detected in systolic/diastolic blood pressure, heart rate or respiration rate in patients treated with

KW-6002 (Hauser et al., 2003), and although anxiety was reported by a small percentage of patients (3 out of 54) treated with the antagonist, the effect was not dose-dependent and only one such case received the highest dose (Hauser et al., 2003). Similarly, the irritability typically observed in $A_{2A}R$ KO mice was not reported by any patient treated with KW-6002 (Bara-Jimenez et al., 2003; Hauser et al., 2003; LeWitt et al., 2008; Stacy et al., 2008; Hauser et al., 2008; Mizuno et al., 2010). The most common adverse effects (AEs) appear to be nausea, aggravation of dyskinesia, dizziness and insomnia, although discontinuation due to AEs was no more frequent than in placebo groups (Bara-Jimenez et al., 2003; Hauser et al., 2003, 2008; LeWitt et al., 2008; Stacy et al., 2008; Factor et al., 2010). Although the clinical effects obtained with KW-6002 are not always statistically significant, they clearly indicate that doses of 20-80 mg/day of this drug reduce the OFF Time by 0.7–1.2 h in PD patients. The drug leads to an increase in ON Time with dyskinesia, though most of this increase can be attributed to non-troublesome dyskinesia. Nevertheless, KW-6002 did not receive the approval by the Food and Drug Administration (FDA) in 2008 despite the positive findings, with the agency expressing concerns as to whether the results from clinical trials supported the clinical utility for KW-6002 and demanding more thorough clinical investigations (www.istradefylline.com/fda.html). Considering the positive clinical results obtained in PD patients, Kyowa decided to perform a further thorough study to elucidate the full potential of KW-6002 as a treatment for PD. Importantly, in PD patients this drug has yet to be tested under similar circumstances to those which revealed positive effects in rodent and primate studies. For example, KW-6002 may be co-administered with a sub-optimal dose of dopamine agonists or L-DOPA, instead of with the optimal doses used so far in clinical trials.

Besides KW-6002, a number of other $A_{2A}R$ antagonists have also entered clinical trials. Accordingly, we will summarize the data recently made available for: preladenant (SCH-420814, **12** from Merck & Co Inc. following its acquisition of Schering-Plough Corp); SYN-115 (**15**: from Roche-Synosia Therapeutics); vipadenant (BIIB014/V2006, **13**: from Vernalis plc-Biogen Idec); and ST-1535 (**14**: from Sigma-Tau. Pinna, 2009; Shah & Hodgson, 2010). Other companies are also working in this field (Adenosine Therapeutics, Palo Biofarma, Neurocrine Biosciences, Almirall Prodesfarma, and Lundbeck) but have yet to disclose significant information related to specific drug development programmes (Pinna, 2009; Shah & Hodgson, 2010).

7.2. Preladenant

Preladenant (previously known as SCH-420814: **12**) is a non-xanthine adenosine $A_{2A}R$ antagonist currently under development for PD treatment by Merck & Co Inc. (following its acquisition of Schering-Plough Corp). It was derived from the well-known SCH-58261 (**10**), a pharmacological tool widely used to characterize the $A_{2A}R$ subtype (Neustadt et al., 2007). Preladenant has good oral bioavailability, it is very selective for $A_{2A}R$ with good pharmacokinetic (PK) properties, and it has excellent *in vivo* activity against parkinsonian symptoms (Neustadt et al., 2007; Hodgson et al., 2009).

A phase I positron emission tomography (PET) trial in healthy humans using the [^{11}C]SCH-442416 (**11**) radiotracer has been undertaken to correlate plasma concentration of preladenant (after 10, 50 and 200 mg) and neural-mediated effects. Accordingly, receptor occupancy in the human brain was maximal at low concentrations of preladenant and the duration occupancy was dose-dependent (Brooks et al., 2009), which supports the possibility of developing preladenant under a twice daily (BID) administration regime. In parallel, the efficacy of the molecule was initially investigated in two phase IIa studies that employed short-term administration of this compound (BID, dosing over 1-3 days), concomitant to L-DOPA, and measuring motor function with the UPDRS motor score. These phase IIa studies

demonstrated that short-term treatment with preladenant plus L-DOPA produced an improvement in motor function in PD patients (Hunter, 2006).

A more extensive international phase II clinical trial (P04501) on preladenant as adjunctive therapy evaluated the efficacy and safety of four different doses (1, 2, 5 or 10 mg BID, 12 weeks) in patients with moderate-to-severe PD that experienced dyskinesia and motor fluctuations (Hauser et al., 2011). All patients were on a stable regime of standard treatments with L-DOPA and other adjunctive medications, such as dopamine agonists and/or entacapone. At doses of 5 and 10 mg BID, preladenant was significantly more effective in reducing the OFF Time than the placebo. In addition, at both doses preladenant significantly increased the ON Time without producing a proportional overall increase in troublesome or non-troublesome dyskinesia, (Hauser et al., 2011).

A further phase II trial (P05175), a 36 week extension to the P04501 trial, was undertaken to assess the long-term safety of preladenant at a dose of 5 mg BID administered in combination with L-DOPA or dopamine agonists (Pinna, 2009; Shah & Hodgson, 2010). The results of this phase II trial in moderate-to-severe PD patients who participated in the main study (P04501) have not yet been disseminated. In addition, a phase II trial (P06402) to administer preladenant (2, 5 or 10 mg BID, 12 weeks) in combination with L-DOPA in Japanese patients with moderate-to-severe PD is currently at the recruitment stage (www.clinicaltrials.gov/ct2/results?term=preladenant). Moreover, four phase III trials (P05664, P04938, P07037 and P06153) with preladenant (2, 5 and 10 mg BID) are underway to evaluate its efficacy in early PD patients as monotherapy over 52 weeks (www.clinicaltrials.gov/ct2/results?term=P05664) in moderate-to-severe PD patients, as adjunctive therapy for 12 weeks (www.clinicaltrials.gov/ct2/results?term=P04938;www.clinicaltrials.gov/ct2/results?term=P07037) and, a 40 week extension of the P04938 and P07037 studies (www.clinicaltrials.gov/ct2/results?term=P06153). From the information available to date from trials, preladenant has been demonstrated to be safe and well tolerated at all doses, with a similar incidence of AEs and discontinuation rates between groups that received preladenant or the placebo (Hauser et al., 2011). A small increase in systolic and diastolic blood pressure was initially observed in patients administered preladenant groups, but this returned to baseline values when measured from 2 to 12 weeks after beginning the treatment (Hauser et al., 2011). Indeed, no dose-dependent effect on blood pressure was detected upon visual inspection of the data. The most frequently AEs reported were worsening PD, dyskinesia and somnolence, which arose at approximately the same frequency in preladenant and placebo groups. There was no clinically significant drug effect on pulse, respiration, or other laboratory and ECG parameters (Hauser et al., 2011).

7.3. SYN-115

Another promising, potent and selective non-xanthine $A_{2A}R$ antagonist is SYN-115 (**15**), which is under development to treat PD by Synosia Therapeutics (acquired in February 2011 by Biotie Therapies) and UCB Pharma. SYN-115 was originally developed by Roche and licensed to Synosia in 2007 (www.biotie.com/en/recearch_and_development/central_nervous_system_disorders/syn115). On the basis of very promising results obtained in preclinical studies, the company decided to perform a phase IIa study with SYN-115 in patients with mild-to-moderate PD. In this study, administration of oral SYN-115 (20 or 60 mg BID, one week), alone or in combination with an infusion of low dose of L-DOPA, was evaluated using a number of techniques, including functional magnetic resonance imaging (fMRI, a tool to rapidly evaluate the pharmacodynamic effects of drugs in the brain), and clinical ratings such as the UPDRS motor scores and tapping speed (Black et al., 2010a, 2010b). SYN-115 produced a dose-responsive decrease in cerebral blood flow in regions of the brain known to be

sensitive to drugs used to treat PD (Black et al., 2010a). Moreover, at 60 mg BID this compound significantly improved tapping speed, both with and without a sub-therapeutic infusion of L-DOPA (Black et al., 2010b). Compared to the placebo, total UPDRS motor scores fell by 20% with SYN-115 when administered with L-DOPA (Black et al., 2010b). Moreover, when considered individually, 10 of 13 items were better with SYN-115 than with placebo; in particular, there were significant improvement in two UPDRS measures of bradykinesia (finger tapping and rapidly alternating hand movements: Black et al., 2010b). These effects were less efficacious at a dose of 20 mg. SYN-115 was well tolerated at both doses and no serious AEs occurred (Black et al., 2010b). On the basis of these promising results, Biotie and UCB are planning to start a phase IIb study over a 12-week treatment period to evaluate the effects of four doses of SYN-115 versus placebo as a L-DOPA adjunctive therapy in PD patients experiencing “wearing off” (www.biotie.com/en/recearch_and_development/central_nervous_system_disorders/syn115).

7.4. Vipadenant

The non-xanthine compound vipadenant (**13**), previously known as BIIB014/V2006, was synthesized by Vernalis plc, who have an agreement with Biogen Idec to develop and commercialise the drug. On the basis of its pharmacological and PK profile, vipadenant was selected for clinical antiparkinsonian evaluation (Gillespie et al., 2009) and indeed, this compound has a high affinity and good selectively for $A_{2A}R$, as well as good oral bioavailability, a long plasma half-life and good brain penetration (Gillespie et al., 2009). These preclinical PK results have been confirmed in healthy humans, showing that vipadenant is appropriate for further development as a single daily treatment (He et al., 2010). Moreover, a PET receptor occupancy study in humans demonstrated that vipadenat is delivered to the brain and that $A_{2A}R$ occupancy is related to both dose and plasma levels (Brooks et al., 2010).

The pharmacological efficacy and safety of vipadenant as an antiparkinsonian drug were investigated in two clinical phase II trials. In the first phase II trial, oral administration of vipadenant (daily, 8 weeks) showed dose-dependent efficacy as an adjunct therapy in association with the habitual L-DOPA treatment of patients with moderate-to-severe PD displaying motor fluctuations; the treatment increased the total ON Time without troublesome dyskinesia and decreasing the OFF Time (Papapetropoulos et al., 2010a). The second phase II study evaluated the effect of vipadenant as monotherapy in patients with early-stage PD (Pinna, 2009), demonstrating that this compound produces a clinically relevant decrease in UPDRS motor scores in a dose-dependent manner (Pinna, 2009). Moreover, Biogen Idec reported that vipadenant was well tolerated in phase II clinical trials in which there was a low incidence of AEs in vipadenant-treated PD patients (Papapetropoulos et al., 2010b). Despite these promising results in phase II clinical studies, development of vipadenant was discontinued in June 2010 (www.vernalis.com/media-centre/latest-releases/2010-releases/584), based on a review of preclinical toxicology findings. As an alternative approach, the two companies are advancing preclinical studies of V81444 to file a phase I clinical trial in 2011. V81444 is considered by the pharmaceutical company as a “next-generation compound” designed to address the chemical structural liability that may have led to the concerns regarding the toxicity of vipadenant (www.vernalis.com/media-centre/latest-releases/596).

7.5. ST-1535

ST-1535 (**14**) was developed by Sigma-Tau with the aim of generating new non-xanthine $A_{2A}R$ antagonists on the basis of $A_{2A}R$ ligand structure-function information (Minetti et al., 2005). After several preclinical studies in rodent and non-human primate models of PD, in

which ST-1535 displayed clear efficacy as an antiparkinsonian drug (Pinna, 2009), a phase I clinical study was designed to ascertain the safety and tolerability of the compound, as well as the most convenient dose. All single doses of ST-1535 (50, 100, 200, 300 and 450 mg) were generally well tolerated and no haematological, biochemical or urinary laboratory abnormalities noted. Based on results of this ongoing phase I clinical study (www.sigma-tau.it/eng/areediricerca.asp), an evaluation of the safety and PK profile of ST-1535 at multiple doses (50, 100, 150 and 200 mg/day, two weeks) has been planned. Moreover, the company has started to investigate the antiparkinsonian activity of two metabolites of ST-1535, ST-3932 and ST-4206, which have good efficacy in rodent models of PD (Vertecchi et al., 2010).

7.6 Future directions

The positive clinical effects displayed by these $A_{2A}R$ antagonists strongly supports the introduction of anti-$A_{2A}R$ medications in the management of parkinsonian patients. Indeed, the observed reduction of L-DOPA OFF Time is of particular interest, since “wearing off” (progressive shortening of L-DOPA motor effects) is one of the major disadvantages of long-term use of this drug. Nevertheless, further studies are required to improve management with $A_{2A}R$ antagonists, particularly in combination with a sub-optimal dose of L-DOPA, as suggested by preclinical trials. Additional results from patients with early stage of PD are expected to elucidate whether $A_{2A}R$ antagonists are suitable for administration as monotherapy against motor symptoms. Finally, evaluation of these drugs in patients devoid of L-DOPA motor complications should be performed in order to assess in more detail the effect of $A_{2A}R$ antagonists on the onset and progress of human dyskinesia.

8. Rationale for developing safer and more effective antiparkinsonian drugs based on targeting $A_{2A}R$ heteromers

8.1. Targeting striatal pre- or postsynaptic $A_{2A}Rs$

The powerful capacity of presynaptic $A_{2A}Rs$ to modulate striatal glutamate release was first demonstrated through *in vivo* microdialysis experiments (Popoli et al., 1995), which revealed that striatal perfusion of an $A_{2A}R$ agonist produced a very pronounced increase in the basal concentrations of extracellular striatal glutamate. Similarly, intra-striatal perfusion of an $A_{2A}R$ antagonist through a microdialysis probe significantly counteracted striatal glutamate release induced by cortical electrical stimulation in the orofacial premotor cortex (Quiroz et al., 2009). Strikingly, an unexpected finding was that this counteracting of glutamate release was accompanied by a complete impairment of the jaw movements induced by the cortical electrical stimulation, demonstrating the crucial role of presynaptic $A_{2A}Rs$ in the control of cortico-striatal glutamatergic neurotransmission. By combining cortical electrical stimulation and recording of EMG activity of the mastication muscles, a Power Correlation Coefficient (PCC) can be used as a quantitative *in vivo* measure of cortico-striatal neurotransmission (Quiroz et al., 2009). PCC was shown to be significantly diminished by systemic administration of an $A_{2A}R$ receptor antagonist in a dose dependent manner. Therefore, the PCC could be used to screen the presynaptic effect of $A_{2A}R$ antagonists.

A recent surprising yet fundamental finding is that several $A_{2A}R$ antagonists previously considered to be pharmacologically similar in fact present different striatal pre- and postsynaptic profiles (Orrú et al., 2011). Six compounds already known as selective $A_{2A}R$ antagonists were first screened for their ability to block striatal pre- and postsynaptic $A_{2A}Rs$ in *in vivo* models. Locomotor activation was used to evaluate postsynaptic activity while counteracting the PCC was used to determine presynaptic activity (see above). SCH-442416 (**11**) and KW-6002 (**5**) preferentially acted pre- and postsynaptically, respectively, and four

compounds had mixed pre-postsynaptic profiles: MSX-3 (**7a**), preladenant (**12**), SCH-58261 (**10**) and ZM-241385 (**9**). The combination of *in vivo* microdialysis with cortical electrical stimulation was used as an additional means to evaluate presynaptic activity of $A_{2A}R$ antagonists *in vivo*. In agreement with its preferential presynaptic profile, SCH-442416 (**11**) counteracted striatal glutamate release induced by cortical stimulation at a dose that strongly counteracted the PCC but did not induce locomotor activation. In accordance with its preferential postsynaptic profile, KW-6002 (**5**) did not modify striatal glutamate release induced by cortical stimulation at a dose that produced pronounced locomotor activation but did not counteract PCC.

Importantly, at least some of the pharmacological differences between $A_{2A}R$ antagonists may be explained by the ability of pre- and postsynaptic $A_{2A}Rs$ to form different receptor heteromers with A_1Rs and D_2Rs, respectively (Orrú et al., 2011). Differences in the affinity of these compounds for different $A_{2A}R$ heteromers were assessed in cells stably expressing $A_{2A}Rs$, $A_{2A}R$-D_2R heteromers or A_1R-$A_{2A}R$ heteromers by radioligand-binding. Co-expression with A_1R did not significantly modify the affinity of $A_{2A}R$ for the different ligands, although co-expression with D_2R decreased the affinity of all compounds, with the exception of KW-6002 (**5**: Orrú et al., 2011). The structural changes in $A_{2A}R$ induced by heteromerization with D_2R were not only detected by antagonists but also by agonist binding. Indeed, the affinity of the selective $A_{2A}R$ agonist CGS-21680 was reduced in cells co-transfected with D_2Rs. In attempting to explain the differential effects of SCH-442416 (**11**) observed *in vivo*, it is interesting to note that this compound in particular exhibited a much higher affinity for the $A_{2A}R$ in a presynaptic versus postsynaptic-like context. In fact, the affinity of $A_{2A}R$ for SCH-442416 (**11**) in cells expressing $A_{2A}R$-D_2R heteromers was markedly reduced (40-fold higher B_{50} values in competitive-inhibition experiments with $[^3H]$ZM-241385 in cells expressing $A_{2A}R$-D_2R than A_1R-$A_{2A}R$ heteromers).

The decrease in affinity upon co-expression with D_2R was much less pronounced for ZM-241385, SCH-58261 (**10**), MSX2 **(7)** or preladenant, for which the affinity fell 2-9 fold (Orrú et al., 2011). Considering that these $A_{2A}R$ antagonists behaved qualitatively similar to the $A_{2A}R$ agonist CGS-21680 in terms of binding to A_1R-$A_{2A}R$ and $A_{2A}R$-D_2R heteromers, it was expected that these four compounds would compete equally for the binding of the endogenous agonist at pre- and at postsynaptic sites. This would fit with the *in vivo* data, which showed that these compounds do not prefer pre-postsynaptic profiles. However, KW-6002 (**5**) was the only antagonist whose affinity was not significantly different in cells expressing $A_{2A}R$, A_1R-$A_{2A}R$ heteromers or $A_{2A}R$-D_2R heteromers. Thus, KW-6002 (**5**) exhibited the best relative affinity for $A_{2A}R$-D_2R heteromers of all compounds, which may partially explain its preferential postsynaptic profile. Experiments performed with the non-selective adenosine receptor antagonist caffeine also revealed a good correlation between the *in vivo* data and the *in vitro* preference for postsynaptic $A_{2A}R$-containing heteromers. In transfected mammalian cells, the affinity of $A_{2A}R$ for the non-selective adenosine receptor antagonist caffeine did not change when co-transfected with D_2R, although it was significantly decreased (about 10 times) when co-transfected with A_1R (Ciruela et al., 2006). As predicted, caffeine did not significantly reduce PCC at doses that produce pronounced motor activation (Zanoveli et al., in the press).

8.2. $A_{2A}R$ heteromers as targets for drug development

The results described above support the proposal that receptor heteromers may be used as selective targets for drug development, particularly given the very specific neuronal localization of receptor heteromers (even more specific than the receptor subtypes themselves) and the distinct ligand affinity of a receptor depending on its partner (or partners) in the heteromer. Striatal $A_{2A}R$-containing heteromers are particularly interesting targets, and especially relevant for a variety of neuropsychiatric disorders. Blocking

postsynaptic A_{2A}Rs in the enkephalinergic MSNs may be beneficial for PD, as this strategy should decrease the activity of the indirect striatal efferent pathway. Potentiating the effect of L-DOPA or other dopamine receptor agonists on D_2R-mediated signalling in the A_{2A}R-D_2R heteromer would exert beneficial effects. However, it should be noted that blockade of presynaptic A_{2A}Rs (that form heteromers with A_1Rs or not) in glutamatergic terminals contacting dynorphinergic MSNs will decrease glutamatergic transmission through the direct striatal efferent pathway, thereby diminishing motor activity and consequently, the antiparkinsonian efficacy of A_{2A}R antagonists. The most convenient A_{2A}R antagonist to treat Parkinson's disease patients should ideally exhibit a higher affinity for post-synaptic versus pre-synaptic receptors. However, selective blockade of presynaptic A_{2A}R is likely to be useful in dyskinetic disorders such as HD, as well as in obsessive-compulsive disorders and drug addiction. It should also be possible to explore whether "pre-synaptic" A_{2A}R antagonists effectively ameliorate L-DOPA-induced dyskinesia.

The antiparkinsonian activity of KW-6002 (**5**) can be explained mechanistically by heteromer–related findings (Orrú et al., 2011), suggesting that SCH-442416 (**11**) may be useful in the treatment of dyskinetic disorders, obsessive-compulsive disorders and drug addiction. Medicinal chemistry and *in silico* modelling should help elucidate the molecular properties that determine the particular pharmacological profile of SCH-442416 (**11**) and KW-6002 (**5**), which may serve as lead compounds to develop more effective antidyskinetic and antiparkinsonian compounds, respectively.

Acknowledgments

This research was funded within the frame of the Era-NET Neuron programme by the following grants: SAF2008-03229-E (RF), SAF2009-07276 (RF) and SAF2008-03118-E (JLL) from the Spanish Ministry of Science and Innovation; 01EW0911 from the German Federal Ministry for Education and Research (BMBF) Germany (CM); and RC2008MinSal/Era-NET from the Italian Ministry of Health (MTA, AP). Support was also obtained through intramural funds of the National Institute in Drug Abuse (SF).

References

Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol. 2000; 20:131–147. [PubMed: 10696506]

Abbott NJ, Revest PA. Control of brain endothelial permeability. Cerebrovasc Brain Metab Rev. 1991; 3:39–72. [PubMed: 2036300]

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci. 1989; 12:366–375. [PubMed: 2479133]

Alvira D, Yeste-Velasco M, Folch J, Verdaguer E, Canudas AM, Pallas M, Camins A. Comparative analysis of the effects of resveratrol in two apoptotic models: inhibition of complex I and potassium deprivation in cerebellar neurons. Neuroscience. 2007; 147:746–756. [PubMed: 17583434]

Andersson M, Usiello A, Borgkvist A, Pozzi L, Dominguez C, Fienberg AA, Svenningsson P, Fredholm BB, Borrelli E, Greengard P, Fisone G. Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. J Neurosci. 2005; 25:8432–8. [PubMed: 16162925]

Angulo E, Casado V, Mallol J, Canela EI, Vinals F, Ferrer I, Lluis C, Franco R. A1 adenosine receptors accumulate in neurodegenerative structures in Alzheimer disease and mediate both amyloid precursor protein processing and tau phosphorylation and translocation. Brain Pathol. 2003; 13:440–451. [PubMed: 14655750]

Armentero MT, Fancellu R, Nappi G, Bramanti P, Blandini F. Prolonged blockade of NMDA or mGluR5 glutamate receptors reduces nigrostriatal degeneration while inducing selective metabolic changes in the basal ganglia circuitry in a rodent model of Parkinson's disease. Neurobiol Dis. 2006a; 22:1–9. [PubMed: 16289868]

Armentero MT, Levandis G, Nappi G, Bazzini E, Blandini F. Peripheral inflammation and neuroprotection: systemic pretreatment with complete Freund's adjuvant reduces 6-

hydroxydopamine toxicity in a rodent model of Parkinson's disease. Neurobiol Dis. 2006b; 24:492–505. [PubMed: 17023164]

Armentero MT, Sinforiani E, Ghezzi C, Bazzini E, Levandis G, Ambrosi G, Zangaglia R, Pacchetti C, Cereda C, Cova E, Basso E, Celi D, Martignoni E, Nappi G, Blandini F. Peripheral expression of key regulatory kinases in Alzheimer's disease and Parkinson's disease. Neurobiol Aging. 2010 Epub ahead of print.

Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handbook Exp Pharmacol. 2011; 220:33–91.

Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neurol. 2001; 50:56–63. [PubMed: 11456310]

Azdad K, Gall D, Woods AS, Ledent C, Ferré S, Schiffmann SN. Dopamine D2 and adenosine A2A receptors regulate NMDA-mediated excitation in accumbens neurons through A2A-D2 receptor heteromerization. Neuropsychopharmacology. 2009; 34:972–86. [PubMed: 18800071]

Bar-Gad I, Bergman H. Stepping out of the box: information processing in the neural networks of the basal ganglia. Curr Opin Neurobiol. 2001; 11:689–695. [PubMed: 11741019]

Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M, et al. Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. Neurology. 2003; 61:293–296. [PubMed: 12913186]

Barcia C, de Pablos V, Bautista-Hernández V, Sánchez-Bahillo A, Bernal I, Fernández-Villalba E, Martín J, Bañón R, Fernández-Barreiro A, Herrero M. ncreased plasma levels of TNF-alpha but not of IL1-beta in MPTP-treated monkeys one year after the MPTP administration. Parkinsonism Relat Disord. 2005; 11:435–439. [PubMed: 16154791]

Barraco RA, Martens KA, Parizon M, Normile HJ. Adenosine A2a receptors in the nucleus accumbens mediate locomotor depression. Brain Res Bull. 1993; 31:397–404. [PubMed: 8490738]

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A. CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. Nat Neurosci. 2001; 4:702–710. [PubMed: 11426226]

Bibbiani F, Oh JD, Petzer JP, Castagnoli N Jr, Chen JF, Schwarzschild MA, et al. A2A antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. Exp Neurol. 2003; 184:285–294. [PubMed: 14637099]

Bilkei-Gorzo A, Abo-Salem OM, Hayallah AM, Michel K, Müller CE, Zimmer A. Adenosine receptor subtype-selective antagonists in inflammation and hyperalgesia. Naunyn-Schmiedeberg's Arch Pharmacol. 2008; 377:65–76.

Black KJ, Koller JM, Campbell MC, Bandak SI. Quantification of indirect pathway inhibition by the adenosine A2a antagonist SYN115 in Parkinson's disease. J Neurosci. 2010a; 30:16284–16292. [PubMed: 21123574]

Black KJ, Campbell MC, Dickerson W, Creech ML, Koller JM, Chung S, et al. A randomized, double-blind, placebo-controlled cross-over trial of the adenosine 2a antagonist SYN115 in Parkinson disease. Presented at the annual meetings of the American Academy of Neurology, Toronto (Canada). Neurology. 2010b; 74(Suppl 2):A317.

Blandini F. An update on the potential role of excitotoxicity in the pathogenesis of Parkinson's disease. Funct Neurol. 2010; 25:65–71. [PubMed: 20923603]

Blandini F, Nappi G, Greenamyre JT. Subthalamic infusion of an NMDA antagonist prevents basal ganglia metabolic changes and nigral degeneration in a rodent model of Parkinson's disease. Ann Neurol. 2001; 49:525–529. [PubMed: 11310632]

Blandini F, Armentero MT, Fancellu R, Blaugrund E, Nappi G. Neuroprotective effect of rasagiline in a rodent model of Parkinson's disease. Exp Neurol. 2004; 187:455–459. [PubMed: 15144871]

Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007; 8:57–69. [PubMed: 17180163]

Blum D, Galas MC, Pintor A, Brouillet E, Ledent C, Müller CE, Bantubungi K, Galluzzo M, Gall D, Cuvelier L, Rolland AS, Popoli P, Schiffmann SN. A dual role of adenosine A_{2A} receptors in 3-

nitropropionic acid-induced striatal lesions: implications for the neuroprotective potential of A_{2A} antagonists. J Neurosci. 2003; 23:5361–5369. [PubMed: 12832562]

Bové J, Marin C, Bonastre M, Tolosa E. Adenosine A2A antagonism reverses levodopa-induced motor alterations in hemiparkinsonian rats. Synapse. 2002; 15:251–257.

Brambilla R, Cottini L, Fumagalli M, Ceruti S, Abbracchio MP. Blockade of A2A adenosine receptors prevents basic fibroblast growth factor-induced reactive astrogliosis in rat striatal primary astrocytes. Glia. 2003; 43:190–194. [PubMed: 12838511]

Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay JM, Duyckaerts C, Flavell RA, Hirsch EC, Hunot S. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest. 2009; 119:182–192. [PubMed: 19104149]

Brooks DJ, Warrington S, Tendolkar A, Cutler DL, Hunter J. Positron emission tomography (PET) study of preladenant in healthy male subjects. Mov Disorders. 2009; 24(Suppl 1):S257.

Brooks DJ, Papapetropoulos S, Vandenhende F, Tomic D, He P, Coppell A, et al. An open-label, positron emission tomography study to assess adenosine A2A brain receptor occupancy of vipadenant (BIIB014) at steady-state levels in healthy male volunteers. Clin Neuropharmacol. 2010; 33:55–60. [PubMed: 20375654]

Brothers HM, Marchalant Y, Wenk GL. Caffeine attenuates lipopolysaccharide-induced neuroinflammation. Neurosci Lett. 2010; 480:97–100. [PubMed: 20541589]

Bura SA, Nadal X, Ledent C, Maldonado R, Valverde O. A 2A adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. Pain. 2008; 140:95–103. [PubMed: 18768260]

Burgueño J, Blake DJ, Benson MA, Tinsley CL, Esapa CT, Canela EI, Penela P, Mallol J, Mayor F Jr, Lluis C, Franco R, Ciruela F. The adenosine A2A receptor interacts with the actin-binding protein alpha-actinin. J Biol Chem. 2003; 278:37545–52. [PubMed: 12837758]

Canals M, Marcellino D, Fanelli F, Ciruela F, de Benedetti P, Goldberg SR, Neve K, Fuxe K, Agnati LF, Woods AS, Ferré S, Lluis C, Bouvier M, Franco R. Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J Biol Chem. 2003; 278:46741–9. [PubMed: 12933819]

Carriba P, Ortiz O, Patkar K, Justinova Z, Stroik J, Themann A, Müller C, Woods AS, Hope BT, Ciruela F, Casadó V, Canela EI, Lluis C, Goldberg SR, Moratalla R, Franco R, Ferré S. Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacol. 2007; 32:2249–59.

Carriba P, Navarro G, Ciruela F, Ferré S, Casado V, Agnati LF, Cortes A, Mallol J, Fuxe K, Canela EI, Lluis C, Franco R. Detection of heteromerization of more than two proteins by sequential BRET-FRET. Nat Methods. 2008; 5:727–733. [PubMed: 18587404]

Carta AR, Kachroo A, Schintu N, Xu K, Schwarzschild MA, Wardas J, Morelli M. Inactivation of neuronal forebrain A receptors protects dopaminergic neurons in a mouse model of Parkinson's disease. J Neurochem. 2009; 111:1478–1489. [PubMed: 19817968]

Carvey PM, Zhao CH, Hendey B, Lum H, Trachtenberg J, Desai BS, Snyder J, Zhu YG, Ling ZD. 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. Eur J Neurosci. 2005; 22:1158–1168. [PubMed: 16176358]

Casadó V, Cortés A, Mallol J, Pérez-Capote K, Ferré S, Lluis C, Franco R, Canela EI. GPCR homomers and heteromers: a better choice as targets for drug development than GPCR monomers? Pharmacol Ther. 2009; 124:248–57. [PubMed: 19664655]

Castagnoli N Jr, Petzer JP, Steyn S, Castagnoli K, Chen JF, Schwarzschild MA, Van der Schyf CJ. Monoamine oxidase B inhibition and neuroprotection: studies on selective adenosine A2A receptor antagonists. Neurology. 2003; 61:S62–68. [PubMed: 14663013]

Cavalcante JL, Barboza J, Ananthasubramaniam K. Regadenoson is a safe and well-tolerated pharmacological stress agent for myocardial perfusion imaging in post-heart transplant patients. J Nucl Cardiol. 2011 May 28. In the press. Epub ahead of print.

Chavez-Valdez R, Wills-Karp M, Ahlawat R, Cristofalo EA, Nathan A, Gauda EB. Caffeine modulates TNF-alpha production by cord blood monocytes: the role of adenosine receptors. Pediatr Res. 2009; 65:203–208. [PubMed: 19047957]

Chen H, Zhang SM, Hernan MA, Schwarzschild MA, Willett WC, Colditz GA, Speizer FE, Ascherio A. Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. Arch Neurol. 2003; 60:1059–1064. [PubMed: 12925360]

Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D, Moskowitz MA, Fink JS, Schwarzschild MA. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J Neurosci. 1999; 19:9192–9200. [PubMed: 10531422]

Chen JF, Moratalla R, Impagnatiello F, Grandy DK, Cuellar B, Rubinstein M, Beilstein MA, Hackett E, Fink JS, Low MJ, Ongini E, Schwarzschild MA. The role of the D(2) dopamine receptor (D(2)R) in A(2A) adenosine receptor (A(2A)R)-mediated behavioral and cellular responses as revealed by A(2A) and D(2) receptor knockout mice. Proc Natl Acad Sci U S A. 2001a; 98:1970–1975. [PubMed: 11172060]

Chen JF, Pedata F. Modulation of ischemic brain injury and neuroinflammation by adenosine A2A receptors. Curr Pharm Des. 2008; 14:1490–1499. [PubMed: 18537672]

Chen JF, Sonsalla PK, Pedata F, Melani A, Domenici MR, Popoli P, Geiger J, Lopes LV, de Mendonca A. Adenosine A2A receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and “fine tuning” modulation. Prog Neurobiol. 2007; 83:310–331. [PubMed: 18023959]

Chen JF, Xu K, Petzer JP, Staal R, Xu YH, Beilstein M, Sonsalla PK, Castagnoli K, Castagnoli N Jr, Schwarzschild MA. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson’s disease. J Neurosci. 2001b; 21:RC143. [PubMed: 11319241]

Chen X, Gawryluk JW, Wagener JF, Ghribi O, Geiger JD. Caffeine blocks disruption of blood brain barrier in a rabbit model of Alzheimer’s disease. J Neuroinflammation. 2008; 5:12. [PubMed: 18387175]

Chen X, Ghribi O, Geiger JD. Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer’s and Parkinson’s diseases. J Alzheimers Dis. 2010; 20(Suppl 1):S127–141. [PubMed: 20164568]

Ciruela F, Casado V, Rodrigues RJ, Luján R, Burgueno J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortés A, Canela EI, López-Giménez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci. 2006; 26:2080–2087. [PubMed: 16481441]

Clementina M, Giuseppe S. A2A receptor ligands: past, present and future trends. Curr Top Med Chem. 2010; 10:902–922. [PubMed: 20370660]

Collins LE, Galtieri DJ, Collins P, Jones SK, Port RG, Paul NE, Hockemeyer J, Müller CE, Salamone JD. Interactions between adenosine and dopamine receptor antagonists with different selectivity profiles: effects on locomotor activity. Behav Brain Res. 2010; 21:148–155. [PubMed: 20211657]

Cookson M, Bandmann O. Parkinson’s disease: insights from pathways. Hum Mol Genet. 2010; 19(R1):R21–27. [PubMed: 20421364]

Correa M, Wisniecki A, Betz A, Dobson DR, O’Neill MF, O’Neill MJ, et al. The adenosine A2A antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to parkinsonism. Behav Brain Res. 2004; 148:47–54. [PubMed: 14684247]

Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A. Caffeine exposure and the risk of Parkinson’s disease: a systematic review and meta-analysis of observational studies. J Alzheimers Dis. 2010; 20(Suppl 1):S221–238. [PubMed: 20182023]

Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson’s disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am J Epidemiol. 2009; 169:919–926. [PubMed: 19270050]

Cristalli, G.; Müller, CE.; Volpini, R. Recent developments in adenosine A2A receptor ligands. In: Wilson, CN.; Mustafa, SJ., editors. Handbook of Experimental Pharmacology, Adenosine receptors in health and disease. Vol. 193. 2009. p. 59-98.

Crossman AR. Primate models of dyskinesia: the experimental approach to the study of basal ganglia-related involuntary movement disorders. Neuroscience. 1987; 21:1–40. [PubMed: 2955248]

Cunha RA. Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. Purinergic Signal. 2005; 1:111–134. [PubMed: 18404497]

Dai SS, Li W, An JH, Wang H, Yang N, Chen XY, Zhao Y, Li P, Liu P, Chen JF, Zhou YG. Adenosine A2A receptors in both bone marrow cells and non-bone marrow cells contribute to traumatic brain injury. J Neurochem. 2010; 113:1536–1544. [PubMed: 20367749]

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005; 8:752–758. [PubMed: 15895084]

DeLeo JA, Yezierski RP. The role of neuroinflammation and neuroimmune activation in persistent pain. Pain. 2001; 90:1–6. [PubMed: 11166964]

DeLong M. Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 1990; 13:281–285. [PubMed: 1695404]

Doengi M, Deitmer JW, Lohr C. New evidence for purinergic signaling in the olfactory bulb: A2A and P2Y1 receptors mediate intracellular calcium release in astrocytes. Faseb J. 2008; 22:2368–2378. [PubMed: 18310463]

El Yacoubi M, Ledent C, Ménard J, Parmentier M, Costentin J, Vaugeois J. The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. Br J Pharmacol. 2000; 129:1465–1473. [PubMed: 10742303]

Factor S, Mark MH, Watts R, Struck L, Mori A, Ballerini R, et al. A long-term study of istradefylline in subjects with fluctuating Parkinson's disease. Parkinsonism Relat Disord. 2010; 16:423–426. [PubMed: 20338800]

Faucheux BA, Bonnet AM, Agid Y, Hirsch EC. Blood vessels change in the mesencephalon of patients with Parkinson's disease. Lancet. 1999; 353:981–982. [PubMed: 10459912]

Fenu S, Pinna A, Ongini E, Morelli M. Adenosine A2A receptor antagonism potentiates L-DOPA-induced turning behaviour and c-fos expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol. 1997; 321:143–147. [PubMed: 9063681]

Fernandez HH, Greeley DR, Zweig RM, Wojcieszek J, Mori A, Sussman NM. Istradefylline as monotherapy for Parkinson disease: results of the 6002-US-051 trial. Parkinsonism Relat Disord. 2010; 16:16–20. [PubMed: 19616987]

Ferré S, Ciruela F, Woods AS, Lluis C, Franco R. Functional relevance of neurotransmitter receptor heteromers in the central nervous system. Trends Neurosci. 2007; 30:440–446. [PubMed: 17692396]

Ferré S, Goldberg SR, Lluis C, Franco R. Looking for the role of cannabinoid receptor heteromers in striatal function. Neuropharmacol. 2009; 56(Suppl 1):226–34.

Ferré S, Quiroz C, Woods AS, Cunha R, Popoli P, Ciruela F, Lluis C, Franco R, Azdad K, Schiffmann SN. An update on adenosine A2A-dopamine D2 receptor interactions. Implications for the function of G protein-coupled receptors. Curr Pharm Des. 2008; 14:1468–1474. [PubMed: 18537670]

Ferré S, Baler R, Bouvier M, Caron MG, Devi LA, Durroux T, Fuxe K, George SR, Javitch JA, Lohse MJ, Mackie K, Milligan G, Pfleger KDG, Pin J-P, Volkow N, Waldhoer M, Woods AS, Franco R. Building a new conceptual framework for receptor heteromers. Nat Chem Biol. 2009; 5:131–134. [PubMed: 19219011]

Ferré S, Lluis C, Justinova Z, Quiroz C, Orru M, Navarro G, Canela EI, Franco R, Goldberg SR. Adenosine-cannabinoid receptor interactions. Implications for striatal function. Br J Pharmacol. 2010; 160:443–453. [PubMed: 20590556]

Fiebich BL, Biber K, Lieb K, van Calker D, Berger M, Bauer J, Gebicke-Haerter PJ. Cyclooxygenase-2 expression in rat microglia is induced by adenosine A2a-receptors. Glia. 1996; 18:152–160. [PubMed: 8913778]

Filion M, Tremblay L, Bédard PJ. Effects of dopamine agonists on the spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res. 1991; 547:152–161. [PubMed: 1677608]

Finberg JP, Wang J, Bankiewicz K, Harvey-White J, Kopin IJ, Goldstein DS. Increased striatal dopamine production from L-DOPA following selective inhibition of monoamine oxidase B by R(+)-N-propargyl-1-aminoindan (rasagiline) in the monkey. J Neural Transm Suppl. 1998; 52:279–285. [PubMed: 9564628]

Fowler JS, Volkow ND, Wang GJ, Logan J, Pappas N, Shea C, MacGregor R. Age-related increases in brain monoamine oxidase B in living healthy human subjects. Neurobiol Aging. 1997; 18:431–435. [PubMed: 9330975]

Franco R. G protein-coupled receptor heteromers or how neurons can display differently flavoured patterns in response to the same neurotransmitter. Br J Pharmacol. 2009; 158:3–31.

Fredduzzi S, Moratalla R, Monopoli A, Cuellar B, Xu K, Ongini E, et al. Persistent behavioral sensitization to chronic L-DOPA requires A2A adenosine receptors. J Neurosci. 2002; 22:1054–1062. [PubMed: 11826134]

Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev. 1999; 51:83–133. [PubMed: 10049999]

Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev. 1999; 51:83–133. [PubMed: 10049999]

Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. Biochem Pharmacol. 2001; 61:443–448. [PubMed: 11226378]

Fredholm BB, IJzerman A, Jacobson KA, Linden J, Müller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. Pharmacol Rev. 2011; 63:1–34. [PubMed: 21303899]

Fuxe K, Agnati LF, Jacobsen K, Hillion J, Canals M, Torvinen M, Tinner-Staines B, Staines W, Rosin D, Terasmaa A, Popoli P, Leo G, Vergoni V, Lluis C, Ciruela F, Franco R, Ferré S. Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. Neurology. 2003; 61:S19–23. [PubMed: 14663004]

Gatev P, Darbin O, Wichmann T. Oscillations in the basal ganglia under normal conditions and in movement disorders. Mov Disord. 2006; 21:1566–1577. [PubMed: 16830313]

Gillespie RJ, Bamford SJ, Botting R, Comer M, Denny S, Gaur S, et al. Antagonists of the Human A2A Adenosine Receptor. 4. Design, Synthesis, and Preclinical Evaluation of 7-Aryltriazolo[4,5-*d*]pyrimidines. J Med Chem. 2009; 52:33–47. [PubMed: 19072055]

Gomes CV, Kaster MP, Tome AR, Agostinho PM, Cunha RA. Adenosine receptors and brain diseases: Neuroprotection and neurodegeneration. Biochim Biophys Acta. 2010

Grondin R, Bedard PJ, Hadj Tahar A, Gregoire L, Mori A, Kase H. Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology. 1999; 52:1673–1677. [PubMed: 10331698]

Halldner L, Lozza G, Lindström K, Fredholm BB. Lack of tolerance to motor stimulant effects of a selective adenosine A(2A) receptor antagonist. Eur J Pharmacol. 2000; 406:345–354. [PubMed: 11040341]

Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007; 10:1387–1394. [PubMed: 17965659]

Hasko G, Pacher P, Vizi ES, Illes P. Adenosine receptor signaling in the brain immune system. Trends Pharmacol Sci. 2005; 26:511–516. [PubMed: 16125796]

Hauber W, Nagel J, Sauer R, Müller CE. Motor effects induced by a blockade of adenosine A_{2A} receptors in the caudate-putamen. Neuroreport. 1998; 9:1803–1806. [PubMed: 9665604]

Hauser RA, Hubble JP, Truong DD. Randomized trial of the adenosine A(2A) receptor antagonist istradefylline in advanced PD. Neurology. 2003; 61:297–303. [PubMed: 12913187]

Hauser RA, Shulman LM, Trugman JM, Roberts J, Mori A, Ballerini R, et al. Study of istradefylline in patients with Parkinson's disease on levodopa with motor fluctuations. Mov Disord. 2008; 23:2177–2185. [PubMed: 18831530]

Hauser RA, Cantillon M, Pourcher E, Micheli F, Mok V, Onofrj M, et al. Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. Lancet Neurol. 2011; 10(3):221–229. [PubMed: 21315654]

Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci. 2006; 9:1512–1519. [PubMed: 17115040]

He P, Papapetropoulos S, O'Neill GN, Wade A, Kwiatkowski K, Donaldson K. Pharmacokinetic profile of the adenosine A2A receptor antagonist BIIB014 in healthy volunteers. Mov Disorders. 2010; 25(Suppl 2):S298.

Higley MJ, Sabatini BL. Competitive regulation of synaptic Ca2+ influx by D2 dopamine and A2A adenosine receptors. Nat Neurosci. 2010; 13:958–66. [PubMed: 20601948]

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J, Canela EI, Zoli M, Agnati LF, Ibanez CF, Lluis C, Franco R, Ferré S, Fuxe K. Coaggregation, cointernalization and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem. 2002; 277:18091–18097. [PubMed: 11872740]

Hirsch E, Hunot S, Damier P, Faucheux B. Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration? Ann Neurol. 1998; 44(suppl 1):S115–120. [PubMed: 9749582]

Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol. 2009; 8:382–397. [PubMed: 19296921]

Hockemeyer J, Burbiel JC, Müller CE. Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's disease. J Org Chem. 2004; 69:3308–3318. [PubMed: 15132536]

Hodgson RA, Bertorelli R, Varty GB, Lachowicz JE, Forlani A, Fredduzzi S, et al. Characterization of the Potent and Highly Selective A2A Receptor Antagonists Preladenant and SCH 412348 in Rodent Models of Movement Disorders and Depression. J Pharmacol Exp Ther. 2009; 330:294–303. [PubMed: 19332567]

Hodgson RA, Bedard PJ, Varty GB, Kazdoba TM, Di Paolo T, Grzelak ME, et al. Preladenant, a selective A(2A) receptor antagonist, is active in primate models of movement disorders. Exp Neurol. 2010; 225:384–390. [PubMed: 20655910]

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci. 2005; 8:858–859. [PubMed: 15965471]

Hunter, J. SCH 420814: a novel Adenosine A2a antagonist. Exploring Parkinson's Disease and beyond. Presented at International research conference "Targeting adenosine A2A receptors in PD and other CNS Disorders"; Boston, USA. 2006 May 17-19. Available from: http://handle.dtic.mil/100.2/ADA452764

Huyck SB, Wolski K, Cantillon M. Impact of A_{2A} receptor antagonist preladenant on dyskinesia in moderate to severe Parkinson's disease: Post-hoc analysis of dose-finding study. Mov Disorder. 2009; 24(Suppl 1):S266.

Ikeda K, Kurokawa M, Aoyama S, Kuwana Y. Neuroprotection by adenosine A2A receptor blockade in experimental models of Parkinson's disease. J Neurochem. 2002; 80:262–267. [PubMed: 11902116]

Ishizaki T, Chiba H, Kojima T, Fujibe M, Soma T, Miyajima H, Nagasawa K, Wada I, Sawada N. Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood-brain-barrier endothelial cells via protein kinase A-dependent and -independent pathways. Exp Cell Res. 2003; 290:275–288. [PubMed: 14567987]

Jaakola VP, Griffith MT, Hanaon MA, Cherezov V, Chien EY, Lane JR, IJzerman AP, Stevens RC. Science. 2008; 322:1211–1217. [PubMed: 18832607]

Jacobson KA, Gallo-Rodriguez C, Melman N, Fischer B, Maillard M, van Bergen A, van Galen PJ, Karton Y. Structure-activity relationships of 8-styrylxanthines as A_2-selective adenosine antagonists. J Med Chem. 1993; 36:1333–1342. [PubMed: 8496902]

Jenner P. Istradefylline, a novel adenosine A2A receptor antagonist, for the treatment of Parkinson's disease. Expert Opin Investig Drugs. 2005; 14:729–738.

Janusz CA, Berman RF. The A2-selective adenosine analog, CGS 21680, depresses locomotor activity but does not block amygdala kindled seizures in rats. Neurosci Lett. 1992; 141:247–250. [PubMed: 1436642]

Joghataie M, Roghani M, Negahdar F, Hashemi L. Protective effect of caffeine against neurodegeneration in a model of Parkinson's disease in rat: behavioral and histochemical evidence. Parkinsonism Relat Disord. 2004; 10:465–468. [PubMed: 15542005]

Jones PA, Smith RA, Stone TW. Protection against hippocampal kainate excitotoxicity by intracerebral administration of an adenosine A2A receptor antagonist. Brain Res. 1998a; 800:328–335. [PubMed: 9685693]

Jones PA, Smith RA, Stone TW. Protection against kainate-induced excitotoxicity by adenosine A2A receptor agonists and antagonists. Neuroscience. 1998b; 85:229–237. [PubMed: 9607714]

Kachroo A, Irizarry MC, Schwarzschild MA. Caffeine protects against combined paraquat and maneb-induced dopaminergic neuron degeneration. Exp Neurol. 2010; 223:657–661. [PubMed: 20188092]

Kalda A, Yu L, Oztas E, Chen JF. Novel neuroprotection by caffeine and adenosine A(2A) receptor antagonists in animal models of Parkinson's disease. J Neurol Sci. 2006; 248:9–15. [PubMed: 16806272]

Kanda T, Shiozaki S, Shimada J, Suzuki F, Nakamura J. KF17837: a novel selective adenosine A2A receptor antagonist with anticataleptic activity. Eur J Pharmacol. 1994; 256:263–268. [PubMed: 8045270]

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol. 1998; 43:507–513. [PubMed: 9546333]

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Combined use of the adenosine A(2A) antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol. 2000; 162:321–327. [PubMed: 10739638]

Karcz-Kubicha M, Antoniou K, Terasmaa A, Quarta D, Solinas M, Justinova Z, et al. Involvement of adenosine A1 and A2A receptors in the motor effects of caffeine after its acute and chronic administration. Neuropsychopharmacology. 2003; 28:1281–1291. [PubMed: 12700682]

Kase H. The adenosine A_{2A} receptor selective antagonist KW6002: research toward a novel nondopaminergic therapy for Parkinson's disease. Neurology. 2003; 61:S97–S100. [PubMed: 14663020]

Kawaguchi Y, Wilson CJ, Emson PC. Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci. 1990; 10:3421–3438. [PubMed: 1698947]

Ke RH, Xiong J, Liu Y, Ye ZR. Adenosine A2a receptor induced gliosis via Akt/NF-kappaB pathway in vitro. Neurosci Res. 2009; 65:280–285. [PubMed: 19666061]

Knott C, Stern G, Wilkin GP. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci. 2000; 16:724–739. [PubMed: 11124893]

Koga K, Kurokawa M, Ochi M, Nakamura J, Kuwana Y. Adenosine A(2A) receptor antagonists KF17837 and KW-6002 potentiate rotation induced by dopaminergic drugs in hemi-Parkinsonian rats. Eur J Pharmacol. 2000; 408:249–255. [PubMed: 11090641]

Koistinaho M, Koistinaho J. Interactions between Alzheimer's disease and cerebral ischemia--focus on inflammation. Brain Res Brain Res Rev. 2005; 48:240–250. [PubMed: 15850663]

Kortekaas R, Leenders KL, van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, Hendrikse NH. Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann Neurol. 2005; 57:176–179. [PubMed: 15668963]

Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, Deisseroth K, Kreitzer AC. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature. 2010; 466:622–626. [PubMed: 20613723]

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312–318. [PubMed: 8843599]

Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience. 1990; 39:151–170. [PubMed: 2089275]

Lee YC, Chien CL, Sun CN, Huang CL, Huang NK, Chiang MC, Lai HL, Lin YS, Chou SY, Wang CK, Tai MH, Liao WL, Lin TN, Liu FC, Chern Y. Characterization of the rat A2A adenosine receptor gene: a 4.8-kb promoter-proximal DNA fragment confers selective expression in the central nervous system. Eur J Neurosci. 2003; 18:1786–1796. [PubMed: 14622213]

Lerner TN, Horne EA, Stella N, Kreitzer AC. Endocannabinoid signaling mediates psychomotor activation by adenosine A2A antagonists. J Neurosci. 2010; 30:2160–4. [PubMed: 20147543]

Levitt P, Pintar JE, Breakefield XO. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. Proceedings of the National Academy of Sciences of the United States of America. 1982; 79:6385–6389. [PubMed: 6755469]

LeWitt PA, Guttman M, Tetrud JW, Tuite PJ, Mori A, Chaikin P, et al. Adenosine A2A receptor antagonist istradefylline (KW-6002) reduces OFF time in Parkinson's disease: a double-blind, randomized, multicenter clinical trial (6002-US-005). Ann Neurol. 2008; 63:295–302. [PubMed: 18306243]

Li XX, Nomura T, Aihara H, Nishizaki T. Adenosine enhances glial glutamate efflux via A2a adenosine receptors. Life Sci. 2001; 68:1343–1350. [PubMed: 11388687]

Lindskog M, Svenningsson P, Pozzi L, Kim Y, Fienberg AA, Bibb JA, et al. Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. Nature. 2002; 418:774–778. [PubMed: 12181566]

Lorenc-Koci E, Wolfarth S, Ossowska K. Haloperidol-increased muscle tone in rats as a model of parkinsonian rigidity. Exp Brain Res. 1996; 109:268–276. [PubMed: 8738375]

Lozano AM, Lang AE, Lewy R, Hutchinson W, Dostrowsky J. Neuronal recordings in Parkinson's disease patients with dyskinesias induced by apomorphine. Ann Neurol. 2000; 47:S141–S146. [PubMed: 10762141]

Lundblad M, Vaudano E, Cenci MA. Cellular and behavioural effects of the adenosine A2a receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. J Neurochem. 2003; 84:1398–1410. [PubMed: 12614340]

Magistretti PJ. Neuron-glia metabolic coupling and plasticity. J Exp Biol. 2006; 209:2304–2311. [PubMed: 16731806]

Marchetti B, Abbracchio MP. To be or not to be (inflamed)--is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? Trends Pharmacol Sci. 2005; 26:517–525. [PubMed: 16126283]

McGeer P, McGeer E. Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. Alzheimer Dis Assoc Disord. 1998; 12(Suppl 2):S1–6. [PubMed: 9769023]

McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology. 1988; 38:1285–1291. [PubMed: 3399080]

McGeer PL, McGeer EG. Innate immunity, local inflammation, and degenerative disease. Sci Aging Knowledge Environ. 2002; 2002:re3. [PubMed: 14602998]

McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord. 2004; 10(Suppl 1):S3–7. [PubMed: 15109580]

McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. Mov Disord. 2008; 23:474–483. [PubMed: 18044695]

McGeer PL, Yasojima K, McGeer EG. Inflammation in Parkinson's disease. Adv Neurol. 2001; 86:83–89. [PubMed: 11554012]

Melani A, Gianfriddo M, Vannucchi MG, Cipriani S, Baraldi PG, Giovannini MG, Pedata F. The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. Brain Res. 2006; 1073-1074:470–480. [PubMed: 16443200]

Minetti P, Tinti MA, Carminati P, Castorina M, Di Cesare MA, Di Serio S, et al. 2-n-Butyl-9-8-[1,2,3]triazol-2-yl-9H-purin-6-ylamine and analogues as A2A adenosine receptor antagonists. Design, synthesis, and pharmacological characterization. J Med Chem. 2005; 48:6887–6896. [PubMed: 16250647]

Minghetti L. Role of inflammation in neurodegenerative diseases. Curr Opin Neurol. 2005; 18:315–321. [PubMed: 15891419]

Minghetti L, Greco A, Potenza RL, Pezzola A, Blum D, Bantubungi K, Popoli P. Effects of the adenosine A2A receptor antagonist SCH 58621 on cyclooxygenase-2 expression, glial activation, and brain-derived neurotrophic factor availability in a rat model of striatal neurodegeneration. J Neuropathol Exp Neurol. 2007; 66:363–371. [PubMed: 17483693]

Mizuno Y, Hasegawa K, Kondo T, Kuno S, Yamamoto M. Clinical efficacy of istradefylline (KW-6002) in Parkinson's disease: a randomized, controlled study. Mov Disord. 2010; 25:1437–1443. [PubMed: 20629136]

Moller T, Kann O, Verkhratsky A, Kettenmann H. Activation of mouse microglial cells affects P2 receptor signaling. Brain Res. 2000; 853:49–59. [PubMed: 10627307]

Monopoli A, Lozza G, Forlani A, Mattavelli A, Ongini E. Blockade of adenosine A2A receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. Neuroreport. 1998; 9:3955–3959. [PubMed: 9875735]

Morelli M, Carta AR, Jenner P. Adenosine A2A receptors and Parkinson's disease. Handb Exp Pharmacol. 2009; 193:589–615. [PubMed: 19639294]

Morissette M, Samadi P, Hadj Tahar A, Bélanger N, Di Paolo T. Striatal Akt/GSK3 signaling pathway in the development of L-Dopa-induced dyskinesias in MPTP monkeys. Prog Neuropsychopharmacol Biol Psychiatry. 2010; 34:446–54. [PubMed: 20026151]

Mott AM, Nunes EJ, Collins LE, Port RG, Sink KS, Hockemeyer J, Müller CE, Salamone JD. The adenosine A_{2A} antagonist MSX-3 reverses the effects of the dopamine antagonist haloperidol on effort-related decision making in a T-maze cost/benefit procedure. Psychopharmacology. 2009; 204:103–112. [PubMed: 19132351]

Müller CE, Ferré S. Blocking striatal adenosine A_{2A} receptors: a new strategy for basal ganglia disorders. Frontiers in CNS Drug Discov. 2010; 1:304–341.

Müller, CE.; Jacobson, KA. Xanthines as adenosine receptor antagonists. In: Fredholm, BB., editor. Handbook of Experimental Pharmacology, Methylxanthines. Vol. 200. 2011a. p. 151-199.

Müller CE, Jacobson KA. Recent development in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta. 2011b; 1808:1290–1308.

Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature. 2004; 431:195–199. [PubMed: 15356633]

Nagatsu T, Sawada M. Inflammatory process in Parkinson's disease: role for cytokines. Curr Pharm Des. 2005; 11:999–1016. [PubMed: 15777250]

Nakaso K, Ito S, Nakashima K. Caffeine activates the PI3K/Akt pathway and prevents apoptotic cell death in a Parkinson's disease model of SH-SY5Y cells. Neurosci Lett. 2008; 432:146–150. [PubMed: 18201823]

Nambu A. Seven problems on the basal ganglia. Curr Opin Neurbiol. 2008; 18:595–604.

Navarro G, Aymerich MS, Marcellino D, Cortes A, Casado V, Mallol J, Canela EI, Agnati LF, Woods AS, Fuxe K, Lluis C, Lanciego JL, Ferré S, Franco R. Interactions between calmodulin, adenosine A2A and dopamine D2 receptors. J Biol Chem. 2009; 284:28058–28068. [PubMed: 19632986]

Navarro G, Ferré S, Cordomi A, Moreno E, Mallol J, Casadó V, Cortés A, Hoffmann H, Ortiz J, Canela EI, Lluís C, Pardo L, Franco R, Woods AS. Interactions between intracellular domains as key determinants of the quaternary structure and function of receptor heteromers. J Biol Chem. 2010; 285:27346–59. [PubMed: 20562103]

Neustadt BR, Hao J, Lindo N, Greenlee WJ, Stamford AW, Tulshian D, et al. Potent, selective, and orally active adenosine A2A receptor antagonists: Arylpiperazine derivatives of pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines. Bioorg Med Chem Lett. 2007; 17:1376–1380. [PubMed: 17236762]

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005; 308:1314–1318. [PubMed: 15831717]

Nishizaki T, Nagai K, Nomura T, Tada H, Kanno T, Tozaki H, Li XX, Kondoh T, Kodama N, Takahashi E, Sakai N, Tanaka K, Saito N. A new neuromodulatory pathway with a glial contribution mediated via A(2a) adenosine receptors. Glia. 2002; 39:133–147. [PubMed: 12112365]

Nobre HV Jr, Cunha GM, de Vasconcelos LM, Magalhaes HI, Oliveira Neto RN, Maia FD, de Moraes MO, Leal LK, Viana GS. Caffeine and CSC, adenosine A2A antagonists, offer neuroprotection against 6-OHDA-induced neurotoxicity in rat mesencephalic cells. Neurochem Int. 2010; 56:51–58. [PubMed: 19782116]

Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. Hum Mutat. 2010; 31:763–780. [PubMed: 20506312]

Obeso JA, Doríguez MC, DeLong MR. Basal ganglia pathophysiology. A critical review. Adv Neurol. 1997; 74:3–18. [PubMed: 9348398]

Obeso JA, Rodríguez-Oroz MC, Rodríguez M, Lanciego JL, Artieda J, Gonzalo N, Olanow CW. Pathophysiology of the basal ganglia in Parkinson's disease. Trends Neurosci. 2000a; 23:S8–S19. [PubMed: 11052215]

Obeso JA, Rodríguez-Oroz MC, DeLong MR, Olanow CW. Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. Ann Neurol. 2000b; 47:S32–S34.

Oh JD, Chase TN. Glutamate-mediated striatal dysregulation and the pathogenesis of motor response complications in Parkinson's disease. Amino Acids. 2002; 23:133–139. [PubMed: 12373527]

Olanow CW, Hauser RA, Jankovic J, Langston W, Lang A, Poewe W, Tolosa E, Stocchi F, Melamed E, Eyal E, Rascol O. A randomized, double-blind, placebo-controlled, delayed start study to assess rasagiline as a disease modifying therapy in Parkinson's disease (the ADAGIO study): rationale, design, and baseline characteristics. Mov Disord. 2008; 23:2194–2201. [PubMed: 18932271]

Olanow CW, Rascol O, Hauser R, Feigin PD, Jankovic J, Lang A, Langston W, Melamed E, Poewe W, Stocchi F, Tolosa E. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N Engl J Med. 2009; 361:1268–1278. [PubMed: 19776408]

Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb Akinesia in the Rat Parkinson Model: Differential Effects of Dopamine Agonists and Nigral Transplants as Assessed by a New Stepping Test. J Neuroscience. 1995; 15:3863–3875.

Ongini E, Fredholm BB. Pharmacology of adenosine A2A receptors. Trends Pharmacol Sci. 1996; 17:364–372. [PubMed: 8979771]

Orr AG, Orr AL, Li XJ, Gross RE, Traynelis SF. Adenosine A(2A) receptor mediates microglial process retraction. Nat Neurosci. 2009; 12:872–878. [PubMed: 19525944]

Orrú M, Bakešová J, Brugarolas M, Quiroz C, Beaumont V, Goldberg SR, Lluís C, Cortés A, Franco R, Casadó V, Canela EI, Ferré S. Striatal Pre- and Postsynaptic Profile of Adenosine A(2A) Receptor Antagonists. PLoS One. 2011; 6:e16088. [PubMed: 21264319]

Papapetropoulos S, Borgohain R, Kellet M, Giladi N, Tomic D, Coppell A, et al. The adenosine A2A receptor antagonist BIIB014 is effective in improving ON-time in Parkinson's disease (PD) patients with motor fluctuations. Mov Disorders. 2010a; 25(Suppl 2):S305.

Papapetropoulos S, Borgohain R, Kellet M, Giladi N, Tomic D, Coppell A, et al. Safety and tolerability profile of the adenosine A2A receptor antagonist BIIB014 in Parkinson's disease: Pooled analysis of two placebo-controlled 8-week studies. Mov Disorders. 2010b; 25(Suppl 2):S304.

Perry VH, Gordon S. Macrophages and microglia in the nervous system. Trends Neurosci. 1988; 11:273–277. [PubMed: 2465626]

Petzer JP, Castagnoli N Jr, Schwarzschild MA, Chen JF, Van der Schyf CJ. Dual-target-directed drugs that block monoamine oxidase B and adenosine A(2A) receptors for Parkinson's disease. Neurotherapeutics. 2009; 6:141–151. [PubMed: 19110205]

Petzer JP, Steyn S, Castagnoli KP, Chen JF, Schwarzschild MA, Van der Schyf CJ, Castagnoli N. Inhibition of monoamine oxidase B by selective adenosine A2A receptor antagonists. Bioorganic & medicinal chemistry. 2003; 11:1299–1310. [PubMed: 12628657]

Phillis J. The effects of selective A1 and A2a adenosine receptor antagonists on cerebral ischemic injury in the gerbil. Brain Res. 1995:705.

Phillis JW. Adenosine in the control of the cerebral circulation. Cerebrovasc Brain Metab Rev. 1989; 1:26–54. [PubMed: 2701369]

Piallat B, Benazzouz A, Benabid AL. Subthalamic nucleus lesion in rats prevents dopaminergic nigral neuron degeneration after striatal 6-OHDA injection: behavioural and immunohistochemical studies. Eur J Neurosci. 1996; 8:1408–1414. [PubMed: 8758948]

Pierri M, Vaudano E, Sager T, Englund U. KW-6002 protects from MPTP induced dopaminergic toxicity in the mouse. Neuropharmacology. 2005; 48:517–524. [PubMed: 15755479]

Pinna A, Di Chiara G, Wardas J, Morelli M. Blockade of A2a adenosine receptors positively modulates turning behaviour and c-Fos expression induced by D1 agonists in dopamine-denervated rats. Eur J Neurosci. 1996; 8:1176–1181. [PubMed: 8752587]

Pinna A, Fenu S, Morelli M. Motor stimulant effects of the adenosine A(2A) receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse. 2001; 39:233–238.

Pinna A, Volpini R, Cristalli G, Morelli M. New adenosine A2A receptor antagonists: actions on Parkinson's disease models. Eur J Pharmacol. 2005; 512:157–164. [PubMed: 15840400]

Pinna A, Pontis S, Borsini F, Morelli M. Adenosine A2A receptor antagonists improve deficits in initiation of movement and sensory motor integration in the unilateral 6-hydroxydopamine rat model of Parkinson's disease. Synapse. 2007; 61:606–614. [PubMed: 17476684]

Pinna A. Novel investigational adenosine A2A receptor antagonists for Parkinson's disease. Expert Opin Investig Drugs. 2009; 18:1619–1631.

Pinna A, Tronci E, Schintu N, Simola N, Volpini R, Pontis S, et al. A new ethyladenine antagonist of adenosine A(2A) receptors: behavioral and biochemical characterization as an antiparkinsonian drug. Neuropharmacology. 2010; 58:613–623. [PubMed: 19951715]

Pollack AE, Fink JS. Synergistic interaction between an adenosine antagonist and a D1 dopamine agonist on rotational behaviour and striatal c-Fos induction in 6-hydroxydopamine-lesioned rats. Brain Res. 1996; 743:124–130. [PubMed: 9017239]

Popoli P, Betto P, Reggio R, Ricciarello G. Adenosine A2A receptor stimulation enhances striatal extracellular glutamate levels in rats. Eur J Pharmacol. 1995; 287:215–217. [PubMed: 8749040]

Popoli P, Blum D, Domenici MR, Burnouf S, Chern Y. A critical evaluation of adenosine A2A receptors as potentially "druggable" targets in Huntington's disease. Curr Pharm Des. 2008; 14:1500–1511. [PubMed: 18537673]

Popoli P, Pintor A, Domenici MR, Frank C, Tebano MT, Pezzola A, Scarchilli L, Quarta D, Reggio R, Malchiodi-Albedi F, Falchi M, Massotti M. Blockade of striatal adenosine A2A receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. J Neurosci. 2002; 22:1967–1975. [PubMed: 11880527]

Powers KM, Kay DM, Factor SA, Zabetian CP, Higgins DS, Samii A, Nutt JG, Griffith A, Leis B, Roberts JW, Martinez ED, Montimurro JS, Checkoway H, Payami H. Combined effects of smoking, coffee, and NSAIDs on Parkinson's disease risk. Mov Disord. 2008; 23:88–95. [PubMed: 17987647]

Pretorius J, Malan SF, Castagnoli N Jr, Bergh JJ, Petzer JP. Dual inhibition of monoamine oxidase B and antagonism of the adenosine A(2A) receptor by (E,E)-8-(4-phenylbutadien-1-yl)caffeine analogues. Bioorg Med Chem. 2008; 16:8676–8684. [PubMed: 18723354]

Privat A. Astrocytes as support for axonal regeneration in the central nervous system of mammals. Glia. 2003; 43:91–93. [PubMed: 12761872]

Quarta D, Borycz J, Solinas M, Patkar K, Hockemeyer J, Ciruela F, Lluis C, Franco R, Woods AS, Goldberg SR, Ferré S. Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation. J Neurochem. 2004; 91:873–80. [PubMed: 15525341]

Quiroz C, Lujan R, Uchigashima M, Simoes AP, Lerner TN, Borycz J, Kachroo A, Canas PM, Orru M, Schwarzschild MA, Rosin DL, Kreitzer AC, Cunha RA, Watanabe M, Ferré S. Key Modulatory Role of Presynaptic Adenosine A2A Receptors in Cortical Neurotransmission to the Striatal Direct Pathway. TheScientificWorldJournal. 2009; 9:1321–1344.

Raivich G, Jones LL, Werner A, Bluthmann H, Doetschmann T, Kreutzberg GW. Molecular signals for glial activation: pro- and anti-inflammatory cytokines in the injured brain. Acta Neurochir Suppl. 1999; 73:21–30. [PubMed: 10494337]

Randall PA, Nunes EJ, Janniere SL, Stopper CM, Farrar AM, Sager TN, Baqi Y, Hockemeyer J, Müller CE, Salamone JD. Stimulant effects of adenosine antagonists on operant behavior: differential actions of selective A_{2A} and A_1 antagonists. Psychopharmacology. 2011 in press.

Rebola N, Porciúncula L, Lopes L, Oliveira C, Soares-da-Silva P, Cunha R. Long-term effect of convulsive behavior on the density of adenosine A1 and A 2A receptors in the rat cerebral cortex. Epilepsia. 2005; 46:159–165. [PubMed: 15987272]

Reiss AB, Rahman MM, Chan ES, Montesinos MC, Awadallah NW, Cronstein BN. Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. J Leukoc Biol. 2004; 76:727–734. [PubMed: 15197231]

Rite I, Machado A, Cano J, Venero JL. Blood-brain barrier disruption induces in vivo degeneration of nigral dopaminergic neurons. J Neurochem. 2007; 101:1567–1582. [PubMed: 17437543]

Rolls A, Shechter R, Schwartz M. The bright side of the glial scar in CNS repair. Nat Rev Neurosci. 2009; 10:235–241. [PubMed: 19229242]

Rose S, Jackson MJ, Smith LA, Stockwell K, Johnson L, Carminati P, et al. The novel adenosine A2a receptor antagonist ST1535 potentiates the effects of a threshold dose of L-DOPA in MPTP treated common marmosets. Eur J Pharmacol. 2006; 546:82–87. [PubMed: 16925991]

Rose S, Ramsay Croft N, Jenner P. The novel adenosine A2a antagonist ST1535 potentiates the effects of a threshold dose of l-dopa in unilaterally 6-OHDA-lesioned rats. Brain Res. 2007; 1133:110–114. [PubMed: 17196564]

Ross G, Abbott R, Petrovitch H, White L, Tanner C. Relationship between caffeine intake and parkinson disease. JAMA. 2000a; 284:1378–1379. [PubMed: 10989386]

Ross GW, Abbott RD, Petrovitch H, White LR, Tanner CM. Relationship between caffeine intake and parkinson disease. Jama. 2000b; 284:1378–1379. [PubMed: 10989386]

Rothwell N, Allan S, Toulmond S. The role of interleukin 1 in acute neurodegeneration and stroke: pathophysiological and therapeutic implications. J Clin Invest. 1997; 100:2648–2652. [PubMed: 9389726]

Saaksjarvi K, Knekt P, Rissanen H, Laaksonen MA, Reunanen A, Mannisto S. Prospective study of coffee consumption and risk of Parkinson's disease. Eur J Clin Nutr. 2008; 62:908–915. [PubMed: 17522612]

Sagi Y, Mandel S, Amit T, Youdim MB. Activation of tyrosine kinase receptor signaling pathway by rasagiline facilitates neurorescue and restoration of nigrostriatal dopamine neurons in post-MPTP-induced parkinsonism. Neurobiol Dis. 2007; 25:35–44. [PubMed: 17055733]

Salamone JD. Preladenant, a novel adenosine A2*A* receptor antagonist for the potential treatment of parkinsonism and other disorders. IDrugs. 2010; 13:723–731. [PubMed: 20878595]

Salamone JD, Mayorga AJ, Trevitt JT, Cousins MS, Conlan A, Nawab A. Tremulous jaw movements in rats: a model of parkinsonian tremor. Prog Neurobiol. 1998; 56:591–611. [PubMed: 9871939]

Salamone JD, Betz AJ, Ishiwari K, Felsted J, Madson L, Mirante B, et al. Tremorolytic effects of adenosine A2A antagonists: implications for parkinsonism. Front Biosci. 2008; 13:3594–3605. [PubMed: 18508458]

Sauer R, Maurinsh J, Reith U, Fülle F, Klotz K-N, Müller CE. Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. J Med Chem. 2000; 43:440–448. [PubMed: 10669571]

Saura J, Angulo E, Ejarque A, Casado V, Tusell JM, Moratalla R, Chen JF, Schwarzschild MA, Lluis C, Franco R, Serratosa J. Adenosine A2A receptor stimulation potentiates nitric oxide release by activated microglia. J Neurochem. 2005; 95:919–929. [PubMed: 16092928]

Schaddelee MP, Voorwinden HL, van Tilburg EW, Pateman TJ, Ijzerman AP, Danhof M, de Boer AG. Functional role of adenosine receptor subtypes in the regulation of blood-brain barrier permeability: possible implications for the design of synthetic adenosine derivatives. Eur J Pharm Sci. 2003; 19:13–22. [PubMed: 12729857]

Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology. 2000; 39:777–787. [PubMed: 10699444]

Schindler CW, Karcz-Kubicha M, Thorndike EB, Müller CE, Tella SR, Ferré S, Goldberg SR. Role fo central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine A_1 and A_{2A} subtype receptor agonists. Br J Pharmacol. 2005; 144:642–650. [PubMed: 15678095]

Shah U, Hodgson R. Recent progress in the discovery of adenosine A(2A) receptor antagonists for the treatment of Parkinson's disease. Curr Opin Drug Discov Devel. 2010; 13:466–480.

Schapira A. Progress in neuroprotection in Parkinson's disease. Eur J Neurol. 2009; 15(suppl. 1):5–13. [PubMed: 18353131]

Seale TW, Abla KA, Shamim MT, Carney JM, Daly JW. 3,7-Dimethyl-1-propargylxanthine: a potent and selective in vivo antagonist of adenosine analogs. Life Sci. 1988; 43:1671–1684. [PubMed: 3193854]

Sherer TB, Betarbet R, Kim JH, Greenamyre JT. Selective microglial activation in the rat rotenone model of Parkinson's disease. Neurosci Lett. 2003; 341:87–90. [PubMed: 12686372]

Shimo Y, Wichmann T. Neuronal activity in the subthalamic nucleus modulates the release of dopamine in the monkey striatum. Eur J Neurosci. 2009; 29:104–113. [PubMed: 19087163]

Singh K, Singh S, Singhal NK, Sharma A, Parmar D, Singh MP. Nicotine- and caffeine-mediated changes in gene expression patterns of MPTP-lesioned mouse striatum: Implications in neuroprotection mechanism. Chem Biol Interact. 2010; 185:81–93. [PubMed: 20230807]

Singh S, Singh K, Gupta SP, Patel DK, Singh VK, Singh RK, Singh MP. Effect of caffeine on the expression of cytochrome P450 1A2, adenosine A2A receptor and dopamine transporter in control and 1-methyl 4-phenyl 1, 2, 3, 6-tetrahydropyridine treated mouse striatum. Brain Res. 2009; 1283:115–126. [PubMed: 19520064]

Shiozaki S, Ichikawa S, Nakamura J, Kitamura S, Yamada K, Kuwana Y. Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology. 1999; 147:90–95. [PubMed: 10591873]

Simola N, Fenu S, Baraldi PG, Tabrizi MA, Morelli M. Blockade of adenosine A2A receptors antagonizes parkinsonian tremor in the rat tacrine model by an action on specific striatal regions. Exp Neurol. 2004; 189:182–188. [PubMed: 15296848]

Simola N, Fenu S, Baraldi PG, Tabrizi MA, Morelli M. Dopamine and adenosine receptor interaction as basis for the treatment of Parkinson's disease. J Neurol Sci. 2006; 248:48–52. [PubMed: 16780890]

Simola N, Morelli M, Pinna A. Adenosine A2A receptor antagonists and Parkinson's disease: state of the art and future directions. Curr Pharm Des 2008. 2008; 14(15):1475–89.

Smith Y, Charara A, Parent A. Synaptic innervation of midbrain dopaminergic neurons by glutamate-enriched terminals in the squirrel monkey. J Comp Neurol. 1996; 364:231–253. [PubMed: 8788247]

Soria G, Castañé A, Ledent C, Parmentier M, Maldonado R, Valverde O. The lack of A2A adenosine receptors diminishes the reinforcing efficacy of cocaine. Neuropsychopharmacology. 2006; 31:978–87. [PubMed: 16123743]

Soriano A, Ventura R, Molero A, Hoen R, Casadó V, Cortés A, Fanelli F, Albericio F, Lluís C, Franco R, Royo M. Adenosine A2A receptor-antagonist/dopamine D2 receptor-agonist bivalent ligands as pharmacological tools to detect A2A-D2 receptor heteromers. J Med Chem. 2009; 5218:5590–602. [PubMed: 19711895]

Stacy M, Silver D, Mendis T, Sutton J, Mori A, Chaikin P, et al. A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson disease. Neurology. 2008; 70:2233–2240. [PubMed: 18519872]

Stasi MA, Borsini F, Varani K, Vincenzi F, Di Cesare MA, Minetti P, et al. ST 1535: a preferential A2A adenosine receptor antagonist. Int J Neuropsychopharmacol. 2006; 9:575–584. [PubMed: 16867213]

Stelzner TJ, Weil JV, O'Brien RF. Role of cyclic adenosine monophosphate in the induction of endothelial barrier properties. J Cell Physiol. 1989; 139:157–166. [PubMed: 2540209]

Stence N, Waite M, Dailey ME. Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia. 2001; 33:256–266. [PubMed: 11241743]

Stevens B, Porta S, Haak LL, Gallo V, Fields RD. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron. 2002; 36:855–868. [PubMed: 12467589]

Stolp HB, Dziegielewska KM. Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. Neuropathol Appl Neurobiol. 2009; 35:132–146. [PubMed: 19077110]

Stone TW, Behan WM. Interleukin-1beta but not tumor necrosis factor-alpha potentiates neuronal damage by quinolinic acid: protection by an adenosine A2A receptor antagonist. J Neurosci Res. 2007; 85:1077–1085. [PubMed: 17304576]

Surmeier DJ, Mercer JN, Chan SS. Autonomous pacemakers in the basal ganglia: who needs excitatory synapses anyway? Curr Opin Neurobiol. 2005; 15:312–318. [PubMed: 15916893]

Svenningsson P, Nomikos GG, Ongini E, Fredholm BB. Antagonism of adenosine A2A receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate-putamen and nucleus accumbens. Neuroscience. 1997; 79:753–764. [PubMed: 9219939]

Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis. 2010; 37:510–518. [PubMed: 19913097]

Tebano MT, Martire A, Chiodi V, Pepponi R, Ferrante A, Domenici MR, Frank C, Chen JF, Ledent C, Popoli P. Adenosine A2A receptors enable the synaptic effects of cannabinoid CB1 receptors in the rodent striatum. J Neurochem. 2009; 110:1921–1930. [PubMed: 19627447]

Torvinen M, Marcellino D, Canals M, Agnati LF, Lluis C, Franco R, Fuxe K. Adenosine A2A receptor and dopamine D3 receptor interactions: evidence of functional A2A/D3 heteromeric complexes. Mol Pharmacol. 2005; 67:400–7. [PubMed: 15539641]

Tozzi A, de Iure A, Di Filippo M, Tantucci M, Costa C, Borsini F, Ghiglieri V, Giampà C, Fusco FR, Picconi B, Calabresi P. The distinct role of medium spiny neurons and cholinergic interneurons in the D2/A2A receptor interaction in the striatum: implications for Parkinson's disease. J Neurosci. 2011; 31:1850–62. [PubMed: 21289195]

Tronci E, Simola N, Borsini F, Schintu N, Frau L, Carminati P, et al. Characterization of the antiparkinsonian effects of the new adenosine A2A receptor antagonist ST1535: acute and subchronic studies in rats. Eur J Pharmacol. 2007; 566:94–102. [PubMed: 17445798]

Tuppo EE, Arias HR. The role of inflammation in Alzheimer's disease. Int J Biochem Cell Biol. 2005; 37:289–305. [PubMed: 15474976]

Varani K, Vincenzi F, Tosi A, Gessi S, Casetta I, Granieri G, Fazio P, Leung E, MacLennan S, Granieri E, Borea PA. A2A adenosine receptor overexpression and functionality, as well as TNF-alpha levels, correlate with motor symptoms in Parkinson's disease. Faseb J. 2010; 24:587–598. [PubMed: 19776336]

Vellucci SV, Sirinathsinghji DJ, Richardson PJ. Adenosine A2 receptor regulation of apomorphine-induced turning in rats with unilateral striatal dopamine denervation. Psychopharmacology. 1993; 111:383–388. [PubMed: 7870978]

Vertechy, M.; Di Serio, S.; Stasi, MA.; Riccioni, T.; Minetti, P.; Piovesan, P., et al. Caratterizzazione "in vivo" dei metaboliti dell'antagonista dei recettori adenosinici A2a, ST1535, per il trattamento del morbo di Parkinson. Presented at XVII Congresso Nazionale della Società Italiana di NeuroPsicoFarmacologia (SINPF); Cagliari (Italy). 2010 september 22-25. Abstract book pag. 103.Available: www.sinpf.it/prjadmin/images/fckimages/Abstract%20Book%20SINPF%202010(1).pdf

Villanueva-Toledo J, Moo-Puc RE, Góngora-Alfaro JL. Selective A2A, but not A1 adenosine antagonists enhance the anticataleptic action of trihexyphenidyl in rats. Neurosci Lett. 2003; 346:1–4. [PubMed: 12850533]

Vlok N, Malan SF, Castagnoli N Jr, Bergh JJ, Petzer JP. Inhibition of monoamine oxidase B by analogues of the adenosine A2A receptor antagonist (E)-8-(3-chlorostyryl)caffeine (CSC). Bioorg Med Chem. 2006; 14:3512–3521. [PubMed: 16442801]

Vollmann K, Qurishi R, Hockemeyer J, Müller CE. Synthesis and properties of a new water-soluble prodrug of the adenosine A_{2A} receptor antagonist MSX-2. Molecules. 2008; 13:348–359. [PubMed: 18305423]

Voss T, Ravina B. Neuroprotection in Parkinson's disease: myth or reality? Curr Neurol Neurosci Rep. 2008; 8:304–309. [PubMed: 18590614]

Wardas J, Konieczny J, Lorenc-Koci E. SCH 58261, an A(2A) adenosine receptor antagonist, counteracts parkinsonian-like muscle rigidity in rats. Synapse. 2001; 41:160–171. [PubMed: 11400182]

Wardas J. Synergistic effect of SCH 58261, an adenosine A2A receptor antagonist, and L-DOPA on the reserpine-induced muscle rigidity in rats. Pol J Pharmacol. 2003; 55:155–164. [PubMed: 12926542]

Weinreb O, Amit T, Bar-Am O, Youdim MB. Rasagiline: a novel anti-Parkinsonian monoamine oxidase-B inhibitor with neuroprotective activity. Prog Neurobiol. 2010; 92:330–344. [PubMed: 20600573]

Weiss N, Miller F, Cazaubon S, Couraud PO. The blood-brain barrier in brain homeostasis and neurological diseases. Biochim Biophys Acta. 2009; 1788:842–857. [PubMed: 19061857]

Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. Br J Pharmacol. 2007; 150:963–976. [PubMed: 17339843]

Wichmann T, DeLong MR. Functional and pathophysiological models of the basal ganglia. Curr Opin Neurobiol. 2000; 6:751–758. [PubMed: 9000030]

Wittendorp MC, von Frijtag Drabbe Kunzel J, Ijzerman AP, Boddeke HW, Biber K. The mouse brain adenosine A1 receptor: functional expression and pharmacology. Eur J Pharmacol. 2004; 487:73–79. [PubMed: 15033378]

Xiao D, Bastia E, Xu YH, Benn CL, Cha JH, Peterson TS, et al. Forebrain adenosine A2A receptors contribute to L-3,4-dihydroxyphenylalanine-induced dyskinesia in hemiparkinsonian mice. J Neurosci. 2006; 26:13548–13555. [PubMed: 17192438]

Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, Stevens RC. Structure of an agonist-bound human A_{2A} adenosine receptors. Science. 2011; 332:322–327. [PubMed: 21393508]

Xu K, Xu YH, Chen JF, Schwarzschild MA. Caffeine's neuroprotection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity shows no tolerance to chronic caffeine administration in mice. Neurosci Lett. 2002; 322:13–16. [PubMed: 11958832]

Xu K, Bastia E, Schwarzschild M. Therapeutic potential of adenosine A(2A) receptor antagonists in Parkinson's disease. Pharmacol Ther. 2005; 105:267–310. [PubMed: 15737407]

Xu K, Xu YH, Chen JF, Schwarzschild MA. Neuroprotection by caffeine: time course and role of its metabolites in the MPTP model of Parkinson's disease. Neuroscience. 2010; 167:475–481. [PubMed: 20167258]

Yasuda S, Sugiura H, Tanaka H, Takigami S, Yamagata K. p38 Map Kinase Inhibitors as Potential Therapeutic Drugs for Neural Diseases. Cent Nerv Syst Agents Med Chem. 2010

Yong-Kee CJ, Salomonczyk D, Nash JE. Development and Validation of a Screening Assay for the Evaluation of Putative Neuroprotective Agents in the Treatment of Parkinson's Disease. Neurotox Res. 2010

Youdim MB, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. British journal of pharmacology. 2006; 147(Suppl 1):S287–296. [PubMed: 16402116]

Yu L, Huang Z, Mariani J, Wang Y, Moskowitz M, Chen JF. Selective inactivation or reconstitution of adenosine A2A receptors in bone marrow cells reveals their significant contribution to the development of ischemic brain injury. Nat Med. 2004; 10:1081–1087. [PubMed: 15448683]

Yu L, Shen H, Coelho J, Araújo I, Huang Q, Day Y, Rebola N, Canas P, Rapp E, Ferrara J, Taylor D, Müller C, Linden J, Cunha R, Chen J. Adenosine A2A receptor antagonists exert motor and neuroprotective effects by distinct cellular mechanisms. Ann Neurol. 2008; 63:338–346. [PubMed: 18300283]

Zhao C, Ling Z, Newman MB, Bhatia A, Carvey PM. TNF-alpha knockout and minocycline treatment attenuates blood-brain barrier leakage in MPTP-treated mice. Neurobiol Dis. 2007; 26:36–46. [PubMed: 17234424]

Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 2008; 57:178–201. [PubMed: 18215617]

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat Neurosci. 2003; 6:43–50. [PubMed: 12469126]

Abbreviations

AR	adenosine receptor(s)
A_{2A}R	adenosine A_{2A} receptor(s)
AD	Alzheimer's disease
AE	adverse effects
AIMs	abnormal involuntary movements
Akt	protein kinase B
BBB	Blood brain barrier
CB_1R	cannabinoid CB_1 receptor(s)
CNS	central nervous system
DA	dopamine
COX	cyclooxygenase
D_1R	dopamine D_1 receptor(s)
D_2R	dopamine D_2 receptor(s)
L-DOPA	L-3,4-dihydroxyphenylalanine
eGFP	enhanced green fluorescent protein
FDA	Food and Drug Administration
GABA	gamma-amino butyric acid
GDNF	glial-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
GP	*Globus pallidus*
HD	Huntington's disease
KW 6002	istradefylline
LPS	lipopolysaccharide
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
$mGlu_5$R	metabotropic glutamate receptor, subtype number 5
MPP^+	1-methyl-4-phenylpyridinium
MSN	medium spiny neurons
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NECA	5'-N-ethylcarboxamidoadenosine
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate
6-OHDA	6-hydroxydopamine

PCC	Power correlation coefficient
PD	Parkinson's disease
PET	Positron emission tomography
SNc	*substantia nigra pars compacta*
SNr	*substantia nigra pars reticolata*
STN	subthalamic nucleus
TNF	tumor necrosis factor
UPDRS motor	motor unified PD rating scale
UPS	ubiquitin/proteasomal system

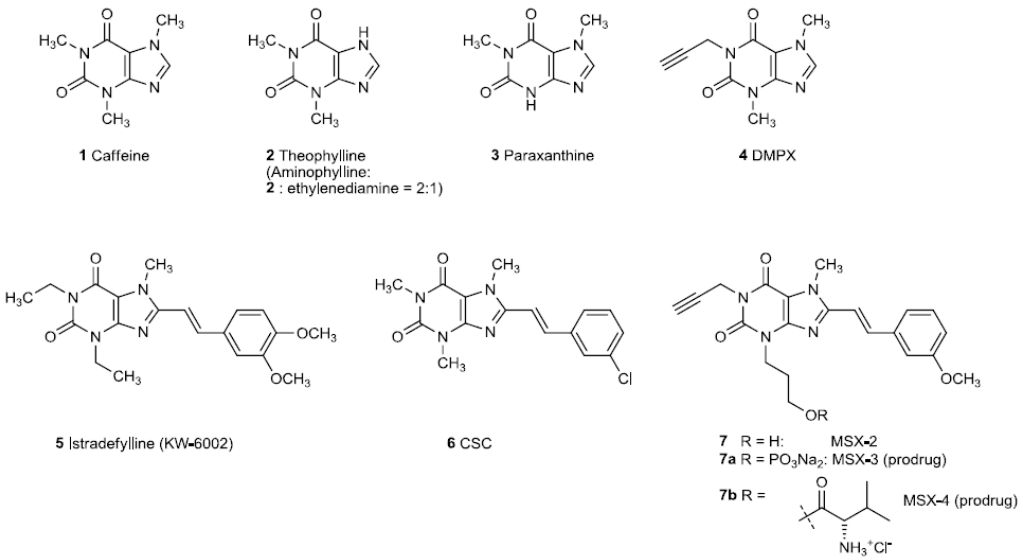

Figure 1. A_{2A} adenosine receptor antagonists: xanthine derivatives

A major metabolite of caffeine in humans, paraxanthine (**3**) is an $A_{2A}R$ antagonist as potent as caffeine that contributes to the *in vivo* activity of caffeine (Arnaud, 2011). A synthetic analogue of caffeine, 3,7-dimethyl-1-propargylxanthine (DMPX, **4**) exhibits a somewhat higher affinity for the A_{2A} versus the A_1 receptor, and has thus been used *in vitro* and *in vivo* as the first "A_{2A}-selective" AR antagonist (Seale et al., 1988). However, DMPX is only very moderately selective with respect to A_1 and it is not selective for the A_{2B} receptor (see Table 1). Truly $A_{2A}R$-selective xanthine derivatives include the 8-styrylxanthine derivatives istradefylline (KW6002, **5**: Kase, 2003), 8-(m-chlorostyryl) caffeine (CSC, **6**: Jacobson et al., 1993), and MSX-2 (**7**: Sauer et al., 2000; Hockemeyer et al., 2004). In contrast to the 8-unsubstituted xanthine derivatives (**1-4**), which exhibit acceptable to good water-solubility, the 8-styrylxanthines (**5-7**) are relatively insoluble in water. Therefore, water soluble prodrugs of MSX-2 have been developed, such as the phosphate prodrug MSX-3 (**7a**: Hockemeyer et al., 2004) and the *L*-valine ester prodrug MSX-4 (**7b**: Vollmann et al., 2008), which represent very valuable pharmacological tools, particularly for *in vivo* studies (*e.g.*, Randall et al., 2011; Collins et al., 2010; Mott et al., 2009; Bilkei-Gorzo et al., 2008; Schindler et al., 2005; Blum et al., 2003; Hauber et al., 1998). These drugs are very water-soluble but readily cleaved by enzymatic hydrolysis. Both compounds can be applied by injection, but are also bioavailable after peroral administration (unpublished results). CSC (**5**) acts as a dual compound, inhibiting monoamine oxidase B (MAO-B) and blocking the $A_{2A}R$ to a similar extent (K_i A_{2A}: 54 nM, K_i MAO-B: 80.6 nM). By contrast, istradefylline (**5**) and MSX-2 (**7**) do not inhibit MAO-B, or only at concentrations that are >100-fold higher than those required for A_{2A} receptor blockade (see Table 1). Istradefylline is the only $A_{2A}R$ -selective xanthine derivative being evaluated in clinical trials (see Figure 2).

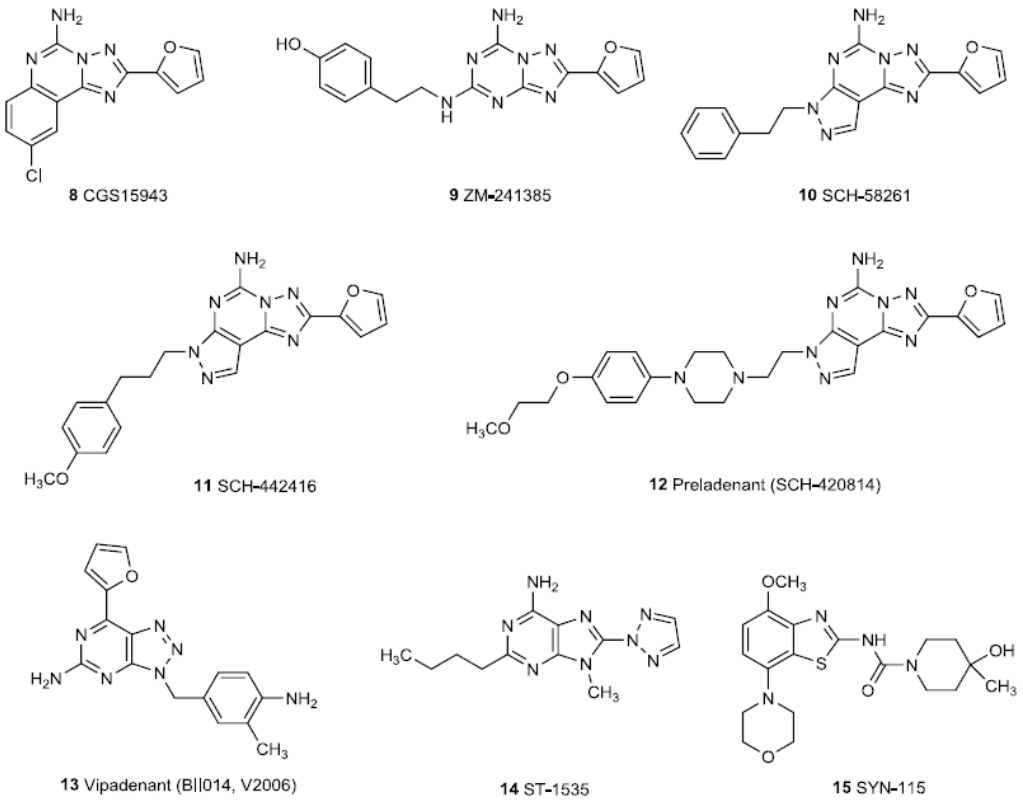

Figure 2. A_{2A}R antagonists: non-xanthine derivatives

Several classes of non-xanthine A_{2A}R antagonists have been developed (see Figure 2 and Table 1). The antagonists frequently used in pharmacological experiments include the non-selective CGS-15943 (**8**), and the A_{2A}R -selective compounds ZM-241385 (**9**) and SCH-58261 (**10**). All of these amino-substituted heterobi- or –tricyclic compounds are structurally related to adenosine, although they lack the ribose sugar moiety (see Figure 2). The X-ray structure of the A_{2A}R protein complexed with antagonist **9** has been obtained, revealing the binding site for antagonists (Jaakola et al., 2008). Very recently an agonist-bound X-ray structure of the A_{2A}R was published showing that the agonist, an adenosine derivative, occupies virtually the same binding site as antagonist **9** (Xu et al., 2011). A further optimized compound derived from SCH-58261 (**10**) has been generated, SCH-442416 (**11**), which exhibits improved selectivity and is used in a ^{11}C-labelled form as a tracer for positron emission tomography (PET: see below). Another analogue of **10** is preladenant (**12**), which is currently being evaluated in clinical trials for the treatment of PD (see below: Salamone, 2010). Two additional aminopurine or aminoazapurine derivatives that have been tested in clinical trials are vipadenant (BII014, V2006, **13** : Gillespie et al., 2009) and ST-1535 (**14**: Stasi et al., 2006). While preladenant is highly A_{2A}R -selective, the latter compounds (**13** and **14**) are less selective (see Table 1). The first selective A_{2A}R antagonist to be tested clinically that is not structurally related to xanthine or adenine was the benzothiazole derivative SYN-115 (**15**: Black et al., 2010). Compound **15** also shows high selectivity for the A_{2A}R.

Table 1

Adenosine receptor affinities of important $A_{2A}R$ antagonists

		K_i (nM)[a]			
		A_1	**A_{2A}**	**A_{2B}**	**A_3**
Xanthines					
1	Caffeine (1,3,7-TrimethylX)	10,700 (h) 44,900 (h) 44,000 (r)	23,400 (h) 9,560 (h) 45,000 (r)	33,800 (h) 10,400 (h) 30,000 (r) 13,000 (m)	13,300 (h) >100,000 (r)
2	Theophylline (1,3-DimethylX)	6,770 (h) 14,000 (r)	1,710 (h) 6,700 (h) 22,000 (r)	9,070 (h) 74,000 (h) 15,100 (r) 5,630 (m)	22,300 (h) 86,400 (h) >100,000 (r) 85,000 (r)
3	Paraxanthine (1,7-DimethylX)	21,000 (r)	32,000 (r)	4,500 (h)	>100,000 (r)
4 DMPX	3,7-Dimethyl-1-propargylX	45,000 (r) 11,000 (r)	16,000 (r) 5,600 (r)	4,130 (h)	>10,000 (r)
5	Istradefylline (KW-6002) (K_i MAO-B = 28,000 nM)[b]	841 (h) 230 (r)	12 (h) 91.2 (h) 2.2 (r) 4.46 (r)	>10,000 (h)	4,470 (h)
6	CSC (K_i MAO-B = 80.6 nM MAO-B)[b]	28,000 (r)	54 (r)	8,200	>10,000 (r)
7	MSX-2	900 (r) 2,500 (h)	8.04 (r) 5.38 (h)	>10,000 (h) 2,900 (h)	>10,000 (h)
Non-xanthines					
8	CGS-15943	3.5 (h) 6.4 (r)	1.2 (h)	32.4 (h) 9.07 (m)	35 (h)

		K_i (nM)[a]			
		A_1	A_{2A}	A_{2B}	A_3
9	ZM-241385	774 (h)	1.6 (h)	75 (h)	743 (h)
10	SCH-58261	725 (h)	5.0 (h)	1110 (h)	1200 (h)
11	SCH-442416	1110 (h)	4.1 (h)	>10,000 (h)	>10,000 (h)
12	Preladenant (SCH-420814)	>1,000 (h)	0.9 (h)	>1,000 (h)	>1,000 (h)
13	Vipadenant (BIIB014, V2006)	68 (h)	1.3 (h)	63 (h)	1,005 (h)
14	ST-1535	71.8 (h)	6.6 (h)	352.3 (h)	>1,000 (h)
15	SYN-115	1,320 (h)	4.9 (h)	nd	nd

[a] h = human; m= mouse; r = rat; a few A_{2B} data are from functional (cAMP) studies (Müller & Jacobson, 2011a; Müller & Jacobson 2011b); nd = no data available

[b] determined with baboon liver enzyme.