Published in final edited form as:
Prog Brain Res. 2010 ; 183: 183–208. doi:10.1016/S0079-6123(10)83010-9.

Pathophysiological roles for purines: adenosine, caffeine and urate

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Abstract

The motor symptoms of Parkinson's disease (PD) are due primarily to the degeneration of the dopaminergic neurons in the nigrostriatal pathway. However, several other brain areas and neurotransmitters other than dopamine such as noradrenaline, 5-hydroxytryptamine and acetylcholine are affected in the disease. Moreover, adenosine because of the extensive interaction of its receptors with the dopaminergic system has been implicated in the in the pathophysiology of the disease. Based on the involvement of these nondopaminergic neurotransmitters in PD and the sometimes severe adverse effects that limit the mainstay use of dopamine-based antiparkinsonian treatments, recent assessments have called for a broadening of therapeutic options beyond the traditional dopaminergic drug arsenal.

In this review we describe the interactions between dopamine and adenosine receptors that underpin the preclinical and clinical rationale for pursuing adenosine A_{2A} receptor antagonists as symptomatic and potentially neuroprotective treatment of PD. The review will pay particular attention to recent results regarding specific A_{2A} receptor-receptor interactions and recent findings identifying urate, the end product of purine metabolism, as a novel prognostic biomarker and candidate neuroprotectant in PD.

1) Localization of adenosine receptors and functional interactions with dopamine receptors

Extensive interactions between adenosine A_1 and A_{2A} receptors and the various dopamine receptors are present in brain at several levels, whereas the interactions between adenosine A_{2A} and dopamine D_2 receptors are restricted within the basal ganglia where they are of particular relevance to the characteristic motor dysfunction of PD.

High densities of adenosine A_{2A} receptors are present in both the ventral and dorsal striatum of rodents and primates, including humans. These receptors colocalize in the striatum with the dopamine D_2 receptor in the dendritic spines of enkephalin-rich striatopallidal GABA

neurons and on glutamatergic terminals (Schiffmann et al., 1991; Rosin et al., 1998). This anatomical framework provides an important structural basis to our understanding of previously discovered A_{2A}/D_2 functional interactions.

In addition, A_{2A} receptors are highly expressed in the globus pallidus (GP), mainly in the neuropil, where their stimulation enhances striatopallidal GABA outflow, and their blockade reduces it (Rosin et al., 1998; Ochi et al., 2000; Shindou et al., 2003). In 6-hydroxydopamine (6-OHDA)-lesioned rats, intrapallidal infusion of A_{2A} receptor antagonists, while not eliciting any motor response per-se, does potentiate motor activity induced by L-DOPA or dopaminergic agonists. This suggests that blockade of pallidal A_{2A} receptors, by reducing extracellular GABA, may stabilize GP activity and in turn subthalamic nucleus (STN) activity (Simola et al., 2006). Therefore, both structures may contribute to the therapeutic action of A_{2A} receptor antagonists.

Adenosine A_{2A} receptors exert an excitatory influence on striatopallidal neurons, in part through their antagonistic effect on dopamine D_2 receptor activation (Fig. 1). The basis of this antagonistic action of adenosine A_{2A} receptors is their ability to decrease the binding affinity of D_2 receptors for dopamine as demonstrated in rat striatal membrane, in human striatal tissue and in different cell lines (Ferré et al., 1991; Diaz-Cabiale et al., 2001; Hillion et al., 2002; Canals et al., 2003). In agreement with these studies, stimulation of adenosine A_{2A} receptors counteracts the D_2 receptor-mediated inhibition of cAMP formation and D_2 receptor-induced intracellular Ca^{2+} responses (Kull et al., 1999; Olah et al., 2000; Salim et al., 2000). Of great importance, A_{2A} receptors exert a strong influence on DARPP-32, a dopamine and cAMP-regulated phosphoprotein, which is expressed at high levels in the GABAergic efferent neurons and is deeply involved in dopamine-mediated signalling (Lindskog et al., 2002) (Fig. 1).

The regulation of dopaminergic signal transduction by A_{2A} receptors is also illustrated by the regulation of CREB activity by A_{2A} receptor stimulation, which increases cAMP formation and in turn phosphorylation of CREB. Selective D_2 receptor agonists dose-dependently counteracted these effects (Kull et al., 1999). Furthermore, a variety of *in vivo* studies support the reciprocal antagonistic influence of A_{2A} and D_2 receptors in induction of immediate early-gene expression (e.g., *c-fos*, *zif/268*, NGFI-B and *jun-B*) (Morelli et al., 1995; Svenningsson et al., 1999; Tronci et al., 2006). Interestingly, the gene expression of striatal A_{2A} receptors and the A_{2A}/D_2 receptor interaction are increased in the dopamine-denervated striatum (Ferré and Fuxe 1992) (Fig. 1). Despite these mentioned findings, it has been shown that stimulation, as well as blockade, of adenosine A_{2A} receptors induces behavioral and biochemical responses in mice lacking dopamine D_2 receptors, suggesting that adenosine A_{2A} receptor actions can occur independently of dopamine signaling (Aoyama et al., 2000).

2) Receptor-receptor interaction: heterodimeric complexes as basis for new antiparkinsonian therapies

An important finding, with respect to A_{2A} receptors, is the formation of functional receptor complexes (receptor mosaics) with other G-protein-coupled receptors (GPCRs). A_{2A} receptors, like many other GPCRs, form both homo and heterodimers (Agnati et al., 2003). This discovery has further increased our understanding of the biology of the A_{2A} receptor with particular emphasis on molecular interactions with receptors for other neurotransmitters such as dopamine D_2, D_3, cannabinoid CB_1 and metabotropic glutamate mGlu5 receptors (Fig. 1). Heterodimerization may have functional and pharmacological consequences; therefore, the presence of heterodimeric complexes has constituted a considerable step

forward in the neurobiology of adenosine, suggesting new ways of modulating neuronal activity by targeting the A_{2A} receptor (Ferré et al., 2009; Fuxe et al., 2003).

Coimmunoprecipitation studies with FRET and BRET analyses have demonstrated the existence of constitutive A_{2A}/D_2 heteromers as well as A_{2A} homodimers within the plasma membrane, indicating less than 10 nm separating the receptors (Hillion et al., 2002; Canals et al., 2003).

Functional implications of the A_{2A}/D_2 intramembrane receptor/receptor interaction through heteromerization, include a decrease in receptor affinity for dopamine agonists acting on D_2 receptors, as well as a reduction of D_2 receptor G protein coupling and signaling. Thus, the essence of this A_{2A}/D_2 receptor heteromerization may be to convert the D_2 receptor into a state of strongly reduced functional activity. The discovery of heteromeric A_{2A}/D_2 complexes has added to the substantial evidence for antagonistic molecular, cellular, electrophysiological and behavioural interactions between A_{2A} and D_2 receptors. These form the basis for antiparkinsonian strategies that simultaneously block adenosine A_{2A} and stimulate dopamine D_2 receptors (Morelli et al., 2007, 2009).

Moreover, evidence for functional A_{2A}/D_3 heteromers has recently been obtained in cotransfected A_{2A}/D_3 cells using FRET. A_{2A} activation reduces the affinity of D_3 agonist binding sites as well as D_3 signaling (Torvinen et al., 2005). It should be noted, however, that while this interaction could provide interesting clues for mediation of ventral striatal (limbic) functions, it might not be relevant for dorsal striatum functions where the D_3 receptor, which is expressed only after dopamine denervation, and the A_{2A} receptor are segregated in different neuronal populations (of striatonigral and striatopallidal neurons respectively) (Bordet et al., 2000).

Although functional properties of multiple receptor heteromers remain to be determined, the interaction of A_{2A} receptors with receptors other than those for dopamine, is also relevant (Fig. 1). Coimmunoprecipitation evidence shows that A_{2A} and mGlu5 receptors form heteromeric complexes and combined stimulation of both of these receptor types, synergistically reduced the affinity of the D_2 receptor agonist binding sites in striatal membranes (Fuxe et al., 2003). These observations were supported by the high degree of A_{2A} and mGlu5 colocalization in primary cultures of striatal neurons and in striatal glutamatergic nerve terminals (Rodrigues et al., 2005). Coactivation of A_{2A} and mGlu5 receptors causes a synergistic interaction at the level of c-*fos* expression and on ERK as well DARPP-32 phosphorylation, indicating a possible role of this heteromeric complex in striatal plasticity (Ferré et al., 2002; Nishi et al., 2003) (Fig. 1). Combined A_{2A} and mGlu5 receptor activation may also produce synergistic cellular effects on striatal output neurons *in vivo*, as demonstrated by a greater than additive increase in GABA release from ventral striatopallidal neurons after local perfusion with both A_{2A} and mGlu5 agonists (Diaz-Cabiale et al., 2002). Similarly, the discovery of functional A_{2A}/mGlu5 receptor interactions and heteromeric A_{2A}/mGlu5 complexes, led to recent findings of a synergistic antiparkinsonian potential of combining A_{2A} and mGlu5 antagonists (Coccurello et al., 2004; Kachroo et al., 2005).

Another interesting interaction that may have important implications for the design of new drugs useful in the treatment of PD, is that of adenosine or dopamine receptors with cannabinoid CB_1 receptors whose presence has been described in basal ganglia, most specifically in GABAergic striatal neurons (Egertova and Elphick, 2000) (Fig. 1). CB_1 receptors colocalize with D_2 and A_{2A} receptors predominantly in the soma and dendrites of the striatopallidal GABA neurons and in corticostriatal glutamate terminals. CB_1-D_2 heteromers as well as CB_1-A_{2A} heteromeric complexes have been described in HEK-293

cell lines (Marcellino et al., 2008; Ferré et al., 2009). Interestingly post-synaptic CB_1 receptor signaling was found to be dependent on A_{2A} receptor activation. Accordingly, blockade of A_{2A} receptors counteracted the motor depressant effects and extracellular field potentials, in corticostriatal neurons, produced by cannabinoid CB_1 agonist (Carriba et al., 2007; Tebano et al., 2009). At the same time, CB1 receptors mediate psychomotor activation by A_{2A} receptors antagonists (Lerner et al., 2010).

Antagonistic CB_1/D_2 interactions have been described at the behavioural level as well. The CB_1 receptor agonist CP 55,940 at a dose that did not change basal locomotion is able to block quinpirole-induced increases in locomotor activity. In addition, not only the CB_1 receptor antagonist rimonabant but also the specific A_{2A} antagonist MSX-3 blocked the inhibitory effect of CB_1 receptor agonists on D_2-like receptor agonist-induced hyperlocomotion (Marcellino et al., 2008). These results find support in the existence of antagonistic CB_1/D_2 receptor-receptor interactions within CB_1/D_2 heteromers in which A_{2A} receptors might also participate (Marcellino et al., 2008) (Fig. 1). Based on this biochemical evidence showing how CB_1 receptors interact with both A_{2A} and D_2 receptors, it has been proposed that these receptors are putative targets for PD.

3) Role of adenosine receptors in neuroprotection

Arresting disease progression at the level of its underlying neuronal degeneration, remains a critical unmet goal of neurotherapeutics for PD. More than a decade of research has suggested that manipulating adenosine neurotransmission might offer a valuable strategy to achieve neuroprotection in PD.

The first studies investigating adenosine and neuroprotection were conducted in models of ischemic and excitotoxic brain injury (reviewed in Fredholm et al., 2005). Under these conditions, increased extracellular adenosine in response to brain injury has been shown to act as a neuroprotectant (Evans et al., 1987; Dux et al., 1990). However, a pro-neurotoxic role of adenosine has also been demonstrated, suggesting that blockade of adenosine receptors may confer neuroprotection across a range of neurodegenerative disorders (Jones et al., 1998; Chen et al., 1999; de Mendonca et al., 2000; Popoli et al., 2002; Melani et al., 2003; Pinna et al., 2010). This apparent paradox reflects the complexity of adenosine transmission, with several receptor subtypes selectively localized in brain areas and uniquely coupled to G proteins and signaling pathways, as described above in this chapter. Moreover, the important role played by adenosine in the immune response in the CNS should be considered as a component of degenerative and protective processes. Hence, the same adenosine receptor subtype expressed in different cell types such as neurons and glia, may mediate opposing effects in response to different neurotoxic insults (Fig. 2).

In PD, the initial suggestion that manipulating adenosine neurotransmission might be beneficial in terms of affecting disease onset or progression came from the epidemiological evidence that consumption of caffeine, a non-specific A_1/A_{2A} receptor antagonist, is associated with a reduced the risk of developing PD (Ascherio et al., 2001; Schwarzschild et al., 2003b). Convergent laboratory studies investigating the effect of caffeine and more specific adenosine receptor antagonists suggested that blockade of the A_{2A} receptor subtype prevents nigrostriatal degeneration in several models of PD (Schwarzschild et al., 2006). Interestingly, epidemiological studies have identified a second purine as another robust inverse risk factor for PD. Blood levels of urate (the end product of adenosine metabolism in humans) is also strongly linked to a reduced risk of PD and, more recently, of its progression, as described below.

Neuroprotection and A_1 adenosine receptors

Preclinical evidence for a role of A_1 receptors in neuroprotection in PD is sparse; therefore this section will focus mostly on a general role of A_1 receptors on glutamate release and toxic cytokine control.

A_1 adenosine receptors are widely distributed throughout the CNS, being expressed both in neuronal and glial cells (Svenningsson et al., 1997; Ochiishi et al., 1999; Daré et al., 2007). Neuronal A_1 adenosine receptors exist in presynaptic terminals as well as in postsynaptic membranes. However the most striking effect of their stimulation is the inhibition of neurotransmitter release, mediated by a reduction of presynaptic calcium influx (Brown et al., 1990; Ochiishi et al., 1999; Masino et al., 2002; Borycz et al., 2007). Although similar effects have been reported for different neurotransmitters, including glutamate, dopamine, acetylcholine and GABA, the inhibition of glutamate release holds the main interest for neurodegeneration. Based on this mechanism, A_1 receptor agonists have been mainly pursued for their potential to protect under conditions characterized by a massive release of glutamate, as occurs in ischemic stroke. Few studies have investigated the effects of A_1 receptor agonism in models of PD. Lau and Mouradian (1993) have shown that an adenosine A_1 agonist was able to prevent the decrease in striatal dopamine levels induced by a single injection of the neurotoxin MPTP. Accordingly, the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxathine (CPX) may have slightly exacerbated striatal MPTP toxicity (Chen et al., 2001). Although the mechanism has not been fully clarified, an inhibition of excessive release of glutamate, which may contribute to MPTP toxicity, might be involved in the protective effect displayed by A_1 receptor agonists in the striatum. In line with this interpretation, blockade of NMDA glutamate receptors achieved a similar protective effect against an acute MPTP insult (Chan et al., 1993). Other clues to A_1-mediated neuroprotection in PD models came from evidence that caffeine pre-treatment protected against methamphetamine-induced nigrostriatal toxicity via an upregulation of A_1 receptors (Delle Donne and Sonsalla, 1994). Accordingly, Alfinito and colleagues have shown that administration of an A_1 antagonist exacerbated dopamine neuron degeneration induced by the mitochondrial inhibitor malonate, although nigral but not striatal A_1 receptors were selectively involved in the effect (Alfinito et al., 2003).

Glial A_1 receptors (Haskó et al., 2008) may play an important role in dopamine neuron demise in PD. The involvement of this receptor in neuroinflammatory responses has been well documented, although direct evidence toward its involvement in neuroinflammation associated with nigrostriatal damage is lacking. In the presence of ischemia and brain injury, extracellular adenosine stimulates glial A_1 receptors, resulting in inhibition of astrocyte proliferation and excessive reactive astrogliosis, as well as increased production of trophic factors, such as nerve growth factor (NGF), S100beta protein and transforming growth factor beta, which in turn may help to protect neurons from injury. Interestingly, the anti-inflammatory cytokine IL-6 enhances the expression of A_1 receptors in astrocytes, suggesting that a self-modulating loop involving A_1 receptors is triggered in presence of a neuronal damage (Schubert et al., 1997; Ciccarelli et al., 2001; van Calker and Biber 2005). Although the topic is still poorly investigated, based on this evidence it is tempting to speculate that a modulation of the astroglial response through the A_1 receptor might be beneficial in PD nigrostriatal degeneration.

Neuroprotection and adenosine A_{2A} receptors

In contrast to the A_1 receptor, the A_{2A} receptor has been consistently implicated as a mediator or modulator of dopaminergic neuron degeneration across a range of laboratory models of PD.

An early suggestion of the neuroprotective potential of A_{2A} receptor blockade in PD came with the demonstration that caffeine can attenuate the loss of striatal dopamine induced by acute MPTP administration in mice (Chen et al., 2001). Caffeine has also been shown to be neuroprotective in other models of PD such as the unilaterally 6-OHDA-lesioned rat (Joghataie et al., 2004) and more recently in a dual pesticide, chronic exposure model (Kachroo et al., 2010), in which repeated systemic administration of paraquat plus maneb leads to degeneration of nigral dopaminergic neurons. In addition, two major demethylation metabolites of caffeine, namely theophylline and paraxanthine, both adenosine receptor antagonists themselves, have also been shown to protect against MPTP-induced neurotoxicity in mice (Xu et al., 2010). These consistent findings of neuroprotection by caffeine and its metabolites in multiple models of PD have strengthened the hypothesis that a true protective effect of caffeine is the basis for its inverse association with PD risk in epidemiological studies (see below).

A similar protective effect was observed upon administration of the selective A_{2A} receptor antagonist KW-6002, but not with the A_1 receptor antagonist CPX (Chen et al., 2001; Pierri et al., 2005; Schwarzschild et al., 2006). In line with these results, genetic deletion of A_{2A} receptors prevented the loss of striatal dopamine induced by acute MPTP (Chen et al., 2001). Thereafter, a protective effect by A_{2A} antagonists was reported in a different toxin model of PD, in which 6-OHDA is infused within the rat striatum (Ikeda et al., 2002). In these studies, neuroprotection was assessed as attenuation of the striatal dopamine depletion or of the loss of TH-positive cells in the substantia nigra *pars compacta* (Table 1). Recently, the A_{2A} receptor antagonists SCH-58261 and ANR 94, were shown to prevent the death of nigral dopaminergic neurons induced by subchronic MPTP administration in mice (Carta et al., 2009; Pinna et al., 2010). Therefore, blockade of the A_{2A} receptor seems to confer a functional protection of striatal dopamine transmission, as well as to prevent the loss of nigral dopaminergic neurons induced by neurotoxin exposure.

Despite considerable evidence suggesting the neuroprotective potential of A_{2A} receptor blockade in PD, the underlying mechanism is still a matter of debate. In neurons, A_{2A} adenosine receptors have been identified both pre- and post-synaptically, where they control neurotransmitter release and neuronal stimulation, respectively (Schiffman et al., 1991; Rosin et al., 1998; Svenningsson et al., 1999; Rebola et al., 2005) (Fig. 2). Moreover, cells involved in the neuroinflammatory response such as astroglia, microglia and bone marrow-derived cells all express the A_{2A} receptor (Fiebich et al., 1996; Saura et al., 2005). Since many factors may contribute to neuronal demise in PD, including neuronal pathological processes and chronic neuroinflammation, several mechanisms have been investigated to explain the neuroprotective effect of A_{2A} receptor blockade. Interestingly, recent reports have highlighted that multiple mechanisms, involving either neuronal or glial receptors, may be recruited in different PD models to achieve neuroprotection by A_{2A} receptor blockade (Yu et al., 2008; Carta et al., 2009).

Neuronal A_{2A} receptors and neuroprotection—An alternative strategy employed to complement pharmacological approaches in demonstrating the neuroprotective efficacy of A_{2A} receptor blockade has been offered by targeted gene mutations producing mice lacking this receptor. In general, A_{2A} receptor knockout mice display attenuated brain damage in models of ischemia or excitotoxin-induced brain injury as had been previously observed using traditional pharmacological antagonists (Phillis, 1995; Monopoli et al., 1998; Chen et al., 1999; Pedata et al., 2005). More recently, studies' using a conditional knockout (Cre/*loxP*) system to generate mice with a selective postnatal depletion of forebrain neuronal A_{2A} receptors has contributed to the understanding of cellular mechanisms of A_{2A} receptor-mediated neuroprotection in PD. In a subchronic MPTP model of PD, loss of these A_{2A} receptors fully prevented neurotoxin-induced degeneration of nigral dopaminergic neurons,

endorsing a primary role of neuronal A_{2A} receptors in the neuroprotective effects of A_{2A} antagonists in this model (Carta et al., 2009). However, neuronal A_{2A} receptors seemed to play a minor role in striatal dopamine loss induced by a more acute MPTP intoxication, since Yu et al. (2008) reported that neuronal A_{2A} receptors inactivation did not protect striatal terminals from a one-day, high-dose MPTP exposure (Yu et al., 2008). Interestingly, in this study mice globally lacking A_{2A} receptors were partially protected against striatal MPTP-toxicity. This suggests that blockade solely of neuronal A_{2A} receptors might not be sufficient to prevent neurotoxicity induced by acute MPTP in the striatum, or alternatively that cells other than forebrain neurons, likely glial cells, may play a role in A_{2A}-mediated protection in the striatum (Yu et al., 2008) (Fig 2). Studies of toxin-induced striatal neuron death modeling the core neurodegenerative features of Huntington disease rather than PD have also given opposing results regarding the role of A_{2A} receptors. In corticostriatal slices A_{2A} receptor antagonism reduced the irreversible functional alterations caused by rotenone in striatal neurons, supporting a beneficial role of neuronal A_{2A} receptor blockade against neurotoxic insults (Belcastro et al., 2009). By contrast, Huang et al reported that in a model of acute striatal neuron damage produced by local infusion of the mitochondrial toxin 3-nitropropionic acid (3-NP), selective deletion of A_{2A} receptors on forebrain neurons was unable to protect against neurotoxicity (Huang et al., 2006). In this model global A_{2A} receptor inactivation actually exacerbated 3-NP-induced neurotoxicity. Moreover, the cell-type-specific inactivation of A_{2A} receptors located in bone marrow-derived cells also exacerbated striatal damage (Huang et al., 2006). All together these data, while supporting a neuroprotective outcome of neuronal A_{2A} receptor blockade in PD, highlight the complexities of the roles played by A_{2A} receptors, pointing to distinct actions of cell-type specific receptors in different neurodegenerative conditions.

In neurons, A_{2A} receptors are enriched in the striatum, but have been also described in other basal ganglia nuclei, such as GP, and at lower levels in the substantia nigra (Schiffmann et al., 1991; Cunha et al., 1994; Johansson et al., 1997; Rosin et al., 1998; Brooks et al., 2008).

At the presynaptic level striatal A_{2A} receptors are mostly located on glutamatergic terminals where they modulate the efflux of glutamate (Marchi et al., 2002; Popoli et al., 2002; Melani et al., 2003; Tebano et al., 2004) (Fig 2). It has been extensively demonstrated that antagonism of the A_{2A} receptor protects against ischemic damage and toxin-induced excitotoxicity in the hippocampus or striatum (Jones et al., 1998; Popoli et al., 2002; Melani et al., 2006). Excessive excitotoxic glutamate efflux from the STN to substantia nigral neurons is a component of PD neuropathology, likely contributing to nigral neuron death. Therefore a reduction of glutamate efflux in the substantia nigra, and perhaps in the striatum, might provide a mechanism for protection against dopaminergic degeneration in PD models (Wallace et al., 2007).

It is important to point out that the effect of A_{2A} receptor antagonists on striatal glutamate levels is dependent on the dose and experimental conditions (Tebano et al., 2004). In intact striatum, the A_{2A} antagonist SCH-58261 decreased evoked glutamate efflux at low doses only, whereas it lost this effect at higher doses (Pintor et al., 2001). Moreover local infusion of an A_{2A} antagonist directly into the 6-OHDA-lesioned striatum increased glutamate outflow (Corsi et al., 2003). Tebano and colleagues have shown that in corticostriatal slices A_{2A} antagonists inhibit glutamate outflow, whereas in striatal neurons they amplify excitotoxic mechanisms due to direct NMDA receptor stimulation (Tebano et al., 2004). Therefore, the potential neuroprotective effect mediated by pre-synaptic A_{2A} receptor blockade might emerge specifically at the low-dose range, whereas other mechanisms, likely mediated by post-synaptic A_{2A} receptors, might mask this effect at higher doses. The higher binding affinity displayed by pre- versus post-synaptic A_{2A} receptors supports this concept (Cunha et al., 1996).

An interesting mechanism of adenosine-glutamate interaction that has emerged recently involves cross-talk between adenosine and GDNF receptors, co-localized on striatal glutamatergic nerve endings (Gomes et al., 2009). In rat striatal synaptosomes, GDNF enhanced glutamate release by 13% from corticostriatal terminals, an effect potentiated by A_{2A} agonist CGS21268 and prevented by the A_{2A} receptor antagonist SCH58261 (Gomes et al., 2009). Therefore, it is suggested that A_{2A} receptor blockade would impair GDNF-stimulated increase of corticostriatal glutamate release, providing a beneficial effect on neurodegeneration (Gomes et al., 2009).

Glial A_{2A} receptors and neuroprotection—Neuroinflammation, characterized by reactive astrocytes and activated microglia, is a hallmark of PD and may play a pathogenic role in dopamine neuron degeneration. Classical studies have described activated microglia in the substantia nigra of PD patients, whereas most recent studies have reported a more widespread distribution of activated microglia in the brain, both in early and late stages of the disease, involving pons, basal ganglia, striatum, and frontal and temporal cortex (Gerhard et al., 2006; Mc Geer and McGeer, 2008). Persistent microglial activation leads to elevated levels of glial-derived cytokines and chronic neuroinflammation, which exert neurotoxic effects on highly vulnerable dopaminergic neurons. On the basis of this evidence it appears of great importance that, in addition to neurons, adenosine A_{2A} receptors are located on both microglia (Fiebich et al., 1996; Saura et al., 2005) and astrocytes (Lee et al., 2003) (Fig. 2). Recent reports *in vivo* have shown that A_{2A} receptor antagonists were able to prevent the astroglial and microglial activation induced by acute or subchronic administration of MPTP in mice (Pierri et al., 2005; Carta et al., 2009). These results are in line with that obtained in an *in vivo* model of ischemia, where the specific A_{2A} receptor antagonist SCH58261 inhibited phosphorylation of P38-MAPK (mitogen activated protein kinase), supporting an inhibitory effect of A_{2A} receptor antagonism on the response in microglial cells (Melani et al., 2006). Therefore, an effect on glial cells has been considered as a potential mechanism of neuroprotection by A_{2A} receptor antagonists. However, the effect on glial response observed in different models of neurodegeneration might either result from a direct effect through stimulation of A_{2A} receptors on these cells, or might be secondary to an effect mediated by A_{2A} receptors on neurons.

The molecular effects of glial A_{2A} receptor stimulation have been investigated by a number of *in vitro* studies, leading to somewhat contradictory results and suggesting that receptors located on microglia and astroglial cells might play opposing roles in neurodegeneration. Supporting a beneficial effect of glial A_{2A} receptor blockade, A_{2A} receptor agonist CGS21680 potentiated lipopolysaccharide-induced NO release and NO synthase-II expression by microglial cells in a concentration-dependent manner, whereas an A_{2A} antagonist suppressed this effect (Saura et al, 2005). Accordingly, A_{2A} receptors mediated the induction of cyclooxygenase-2 and nitric oxide synthase in microglia (Fiebich et al., 1996, 1998).

However, A_{2A} receptor agonists inhibited cytokines production by activated microglia (van der Putten et al, 2009). In cultured astroglia, both adenosine kinase inhibitors and the A_{2A} receptor agonist CGS21280 counteracted LPS-induced production of NO (Brodie et al., 1998; Lee et al., 2005). In line with a positive role of A_{2A} receptor blockade, the A_{2A} receptor agonist CGS21680 increased the astrocytic release of glutamate via an A_{2A} receptor/PKA signaling pathway and via inhibition of glutamate transporter GLT-1, resulting in increased synaptic concentrations of this neurotransmitter, and likely in deleterious effects on neurons survival. Most importantly, this effect was inhibited by the A_{2A} receptor antagonist DMPX (Nishizaki, 2004). Furthermore, adenosine and the A_{2A} receptor agonist CPCA increased astrocyte proliferation after brain injury in rat cortex, whereas adenosine antagonists where shown to counteract astrocytic proliferation in both *in*

vitro and *in vivo* models of brain injury (Hindley et al., 1994; Brambilla et al., 2003; Pugliese et al., 2009). Therefore, whereas substantial evidence suggests that manipulating glial A_{2A} receptors might be beneficial in neurodegenerative conditions, their selective stimulation or blockade might be beneficial depending on neurodegenerative conditions and time of intervention (Fig. 2). Moreover, reports on glial A_{2A} receptors manipulation in *in vivo* PD models are currently lacking.

Lately, a new alternative mechanism of neuroprotection has been suggested, involving A_{2A} receptors located on oligodendrocytes. In a stroke model A_{2A} receptor antagonist SCH58261 reduced striatal Olig 2 transcription factor induced by ischemia (Melani et al., 2009).

4) Epidemiology and clinical trials of adenosine antagonists

Caffeine Epidemiology

Case control studies: coffee—Considerable epidemiological and laboratory data have suggested that A_{2A} receptor blockade by caffeine, a nonselective adenosine receptor antagonist, may protect against the underlying neurodegeneration of PD. Drinking caffeinated beverages (coffee and to a lesser extent tea) has emerged as the dietary factor most consistently linked to an altered risk of PD, with greater consumption associated with a reduced risk (Hellenbrand et al., 1996; Fall et al., 1999; Benedetti et al., 2000; Ross et al., 2000; Ascherio et al., 2001; Checkoway et al., 2002; Ragonese et al., 2003; Tan et al., 2003; Hu et al., 2007). In the early 90's case-control studies suggested a reduced risk of developing PD associated with drinking coffee but the reduction either was not statistically significant (Jimenez-Jimenez et al., 1992; Morano et al., 1994), or was significant but partially attributable to the confounding association of smoking in coffee drinkers (Grandinetti et al., 1994) and thus difficult to interpret. Following on from this, larger, case-control studies, using better-matched cohorts demonstrated that even after adjusting for tobacco smoking and other potential confounding factors, the significant inverse relationship between prior coffee drinking exposure and PD remained (Hellenbrand et al., 1996; Fall et al., 1999; Benedetti et al., 2000). These latter studies also specifically investigated dose-response relationships, with increasing coffee consumption (measured in cups per day) associated with decreasing likelihood of having developed PD. In these retrospective analyses PD patients were 4 to 8 times less likely than control subjects to have reported being heavy coffee drinkers in the past. While case control studies have some advantages, weaknesses of these designs for investigating dietary etiology of chronic diseases are well known (Willett, 1998) and include for example difficulty selecting appropriate control subjects and recall bias. The introduction of large cohort prospective investigations (see below) has overcome many of these limitations through follow-up evaluations to determine disease incidence in subjects from a single population in which the exposure in question (i.e., coffee or caffeine consumption) had been reported years earlier.

Case-control studies: tea—Relatively few studies have considered the effect of tea drinking and its association with PD risk. This could be attributed to its low consumption in North America and Europe (Ascherio et al., 2001; Chan et al., 1998). These studies usually (Ho et al., 1989; Hellenbrand et al., 1996; Ayuso-Peralta et al., 1997; Chan et al., 1998; Checkoway et al., 200; Tan et al., 2003, 2007), although not always (Preux et al., 2000), indicated a reduced risk of developing PD amongst frequent tea drinkers. Interestingly, epidemiological studies from China have observed that the prevalence of PD is much lower than in the Caucasian population (Li et al., 1985; Zhang et al., 1993). Barranco et al., 2009 reviewed observational studies that evaluated tea consumption and the risk of PD (11 case-control and 1 cohort) between 1981 and 2003. The studies represented documented cases from North America, Europe and Asia. Amongst the case-control studies, the pooled OR was 0.8 (95% CI 0.71 to 0.90) suggesting that tea consumption is inversely associated with

the risk of PD. It is unclear whether the active ingredient(s) mediating this observed protective effect is caffeine or some biologically active substance(s) present in tea but not coffee.

Cohort studies

Caffeinated beverages—More convincing epidemiological evidence that caffeine as well as coffee consumption are linked to a reduced risk of developing PD has been obtained from the study of prospectively followed, large populations (Ross et al., 2000; Ascherio et al., 2001). Three decades after ~8000 Japanese-American men were enrolled in the Honolulu Heart Program (and provided details of their dietary caffeine consumption), over 100 had gone on to develop PD. Higher initial coffee intake was dose-dependently associated with a reduced incidence of PD, with a 5-fold lower risk amongst those who drank over 24 oz per day (Ross et al., 2000). Confirmation of these findings was provided by two prospective studies of larger, multiethnic populations, namely the Health Professionals Follow-up Study (HPFS) of 50,000 men followed for a period of 10 years, and the Nurses Health Study (NHS) of 90,000 women followed over 16 years (Ascherio et al., 2001). Amongst the men, increased coffee, tea and non-coffee caffeine consumption, as well as total caffeine consumption were all significantly, dose-dependently and negatively correlated with the incidence of subsequent PD. These associations were independent of smoking and other potential confounding factors. By contrast, the rates of consuming *decaffeinated* coffee were unrelated to the risk of PD, implicating caffeine as the component in coffee that is inversely associated with PD risk. Similar findings were observed in 2 separate cohort studies of Finnish men and women free from PD at baseline (Hu et al., 2007; Saaksjarvi et al., 2008). Heavier coffee consumption was associated with a reduced risk of PD even after adjustment for confounding factors. The Finnish population is of particular interest since it exhibits one of the world's highest rates of coffee consumption (Fredholm et al., 1999).

Gender differences (estrogen interactions)—Interestingly, a stark gender difference in how caffeine relates to PD risk emerged from comparison of female and male cohorts within a large protective study of health care workers (Ascherio et al., 2001). Initially, analysis of women in the NHS study revealed no clear relationship between PD and caffeine or coffee intake. This gender difference was consistent with the observations of PD incidence rates in Olmstead County, MN, which were strongly inversely related to prior coffee drinking in men (with a ~17-fold reduction in risk amongst drinkers vs. non drinkers; $p < 0.01$) but did not vary with coffee exposure in women (with a relative risk of 1.0; Benedetti et al., 2000). Ascherio and colleagues gained insight upon stratification of the women by estrogen exposure history. In two separate prospective studies (Ascherio et al., 2003, 2004) they showed that amongst women who did not use postmenopausal estrogens, caffeine was in fact associated with a reduction in the risk of subsequent PD (just as in men). Conversely, for women who had used estrogen replacement caffeine use did not carry a lower risk of PD, suggesting a hormonal basis for the gender difference in caffeine's association with PD.

Interestingly, in contrast to the result from studies by Ascherio and colleagues, the two prospective cohort studies of Finnish populations (Hu et al., 2007; Saaksjarvi et al., 2008) reported no gender differences in the (inverse) relationship between coffee consumption and PD risk. However as Saaksjarvi and colleagues note in their study, the effect of postmenopausal hormone use could not be examined due to small number of users, which was reported to be ~5% of women and markedly less than in the US cohorts. For example in the NHS, a third of the women were currently taking postmenopausal estrogens and 54% reported ever taking them. Thus, the variability in an overall association between caffeine

and PD risk among women between cohorts may be explained by wide variation in their use of postmenopausal estrogen, which appears to complicate the relationship between caffeine and PD.

Recent laboratory experiments have explored the biology that may underlie gender differences in the caffeine-PD link observed in populations with higher rates of estrogen use. Xu et al., 2006 found that the ability of caffeine to attenuate MPTP toxicity in a mouse model of PD was greater in male *versus* female mice, and in ovariectomized *versus* sham-operated female mice. They also demonstrated that chronic estrogen replacement undermined caffeine's protective effect both in male and in ovariectomized female mice, providing direct evidence of a hormonal influence on caffeine's neuroprotective properties in lab animals. The study also implicated a biological basis for the gender difference in the association between caffeine consumption and PD risk. In general, these and other laboratory studies (as reviewed above) support – but do not prove -- the hypothesis that the consistent epidemiological association between caffeine and a reduced risk of PD is causal.

PD progression—Convergent epidemiological and laboratory data support the possibility that dietary caffeine reduces the risk of developing PD. Less well known is the relationship between caffeine intake and the rate of progression of the disease. A preliminary investigation (Schwarzschild et al, 2003a) reported a secondary analysis of the 'Comparison of the agonist pramipexole versus levodopa on the motor complications of PD' (CALM-PD) trial database, which included data over 5 years from 268 participating PD patients (87% of the original CALM-PD cohort). No association was found between caffeine intake (from coffee, tea and soda sources) and rate of PD progression even after adjustment for treatment group, gender, tobacco and alcohol use. Outcome measures assessed progression by change in the Unified Parkinson Disease Rating Scale (UPDRS), or by alteration in the striatal uptake of iodine-123-labeled 2-β-carboxymethoxy-3-β-(4-iodophenyl) tropane ([^{123}I] β-CIT) in the subset of patients that underwent neuroimaging. The study also allowed for assessment of caffeine consumption and dyskinesia development. A trend albeit non-significant toward lower risk of dyskinesias with increasing caffeine intake was observed. The lack of an association between caffeine consumption and rate of subsequent progression was also observed in a NET-PD trial cohort (Simon et al., 2008) monitoring changes in the UPDRS score and likelihood of disease progression to the point of requiring symptomatic therapy as measurable outcomes. These studies do not lend support to a protective benefit of caffeine by PD patients. However, they leave open the possibility that disease progression among individuals who develop PD despite ingesting caffeine at higher levels may be less influenced by any true protective effect it may have.

5) Clinical trials of A_{2A} antagonists for Parkinson's disease

Trials for symptomatic anti-parkinsonian indications

Non-selective adenosine antagonists: caffeine and theophylline—Although caffeine is a non-specific adenosine receptor antagonist that blocks with similar potency at the A_1, A_{2A} and A_{2B} subtypes, (Fredholm et al., 1999) it appears to have its main psychomotor stimulant actions primarily through blockade of CNS A_{2A} receptors (Xu et al., 2004; Yu et al., 2008). Thus the first trials of A_{2A} antagonism in PD may have been a pair of double-blind, placebo-controlled studies of caffeine effects on parkinsonian symptoms of patients taking levodopa or a dopamine agonist in the 1970s (Shoulson et al., 1975; Kartzinel et al., 1976). Both studies found no benefit of caffeine. However, their small size (<10 subjects), clinical heterogeneity (some subjects did not have PD) and extremely high dosing 1-1.5 grams of caffeine daily precludes meaningful conclusions about the symptomatic utility of caffeine for PD from these studies. A more rigorously designed randomized, placebo-controlled trial of caffeine at more pharmacologically informative

doses (100 or 200 mg bid) is currently underway and projects enrollment of some 50 subjects with idiopathic PD and excessive daytime somnolence (McGill University Health Center; clinical trials.gov identifier # NCT00459420). Although its primary outcome focuses on daytime sleepiness, a secondary analysis of parkinsonian motor symptoms is planned using the standard Unified Parkinson's Disease Rating Scale.

Theophylline, a demethylation metabolite of caffeine as well as an anti-asthmatic agent in common use, is also a non-specific adenosine antagonist. Although small open-label trials of theophylline in PD seemed to suggest antiparkinsonian benefit without exacerbation of dykinesias (Mally & Stone, 1994; Kostic et al., 1999), a subsequent double-blind, placebo-controlled trial of theophylline in PD did not clearly demonstrate relief from symptoms (Kulisevsky et al., 2002). However, the power and design of all these trials like the earlier caffeine studies in PD were not sufficient to rule out clinically useful symptomatic effects, as have been suggested by long-standing preclinical studies of motoric enhancement in PD models (Stromberg & Waldeck, 1973).

Selective adenosine A_{2A} antagonists—A clear indication of the promise of adenosine A_{2A} receptor antagonism for PD is the depth of industry investment in programs to develop a variety of xanthine and non-xanthine structure based adenosine receptor antagonists as antiparkinsonian agents. Amongst at least ten publicly announced programs are some five that have entered human studies, with four being now actively pursued through phase II and III clinical trials: istradefylline (aka KW-6002) from Kyowa-Kirin (Kyowa Hakko Kirin Co., 01.15.2009 announcement). Preladenant (aka SCH 420814) from Schering Plough [now Merck] (Schering Plough Co., 11.24.2008 announcement). BIIB014 (aka V2006) from BiogenIdec [licensed from Vernalis] (Papapetropoulos et al., 2010) and Syn-115 from Synosia [licensed from Roche] (Black et al., 2010 a and b).

The early frontrunner amongst these clinical development programs was istradefylline, which had progressed through encouraging phase II trial results (Bara-Jimenez et al., 2003; Hauser et al., 2003; Kase et al., 2003; Hauser et al., 2008; Lewitt et al., 2008; Stacy et al., 2008; Fernandez et al., 2010) to as yet unpublished, reportedly mixed results of phase III studies (Kyowa Hakko Kirin Co., 06.03.2007 announcement) before submission of a New Drug Application (NDA) to US Food and Drug Administration (FDA). However, the FDA in response issued a ‘Non-Approvable letter’ in 2008 based on concerns of the adequacy of overall efficacy (Kyowa Hakko Kirin Co., 02.08.2008 announcement). Since then clinical development of istradefylline has continued, but the company has pulled back from an international program to focus on trials and a potential indication in Japan.

Based on available data for the published phase II trials, istradefylline employed (at once-daily doses from 20-80 mg) as adjunctive therapy in relatively advanced subjects, produced a modest but significant reduction in “off” time (i.e., in motor dysfunction). Of note, these ‘positive’ outcome measures were based on reports of the patients themselves, whereas no significant treatment-induced improvement was observed based on the clinician-scored UPDRS. Although preclinical evidence supporting the original study designs had suggested adjunctive A_{2A} antagonism might assuage PD symptoms without exacerbating dyskinesias (reviewed in Xu et al., 2004), istradefylline treatment in this relatively advanced PD population actually increased dyskinesias in most studies. However when dyskinesias were stratified into “troublesome” and “non-troublesome”, only the latter were significantly increased on the drug.

Recently, istradefylline was also tested as monotherapy for its potential symptomatic benefit in early PD (Fernandez et al., 2010). Despite a trend toward improvement on istradefylline, again the difference between placebo and A_{2A} antagonist-treated groups did not reach

significance for the change in UPDRS score, the primary outcome in this study. Retrospective assessment of why this early A_{2A} antagonist development program fell short in its initial efforts to gain an indication for PD treatment have focused on trial design elements, including dose selection (for both istradefylline and concomitant levodopa) and disease stage of the targeted PD subpopulation (i.e., possibly too advanced in adjunctive trials, though possibly too early in the monotherapy trial).

In addition, none of the trials reports addressed the potential confound of concomitant A_{2A} antagonism by caffeine use, which apparently was neither excluded nor monitored. At doses relevant to typical human consumption, caffeine and more specific A_{2A} antagonists (including istradefylline) bind to striatal A_{2A} receptors *in vivo* to a similar extent (El Yacoubi et al., 2001; Moresco et al., 2005). Given also the well-known psychomotor stimulant properties of caffeine and its continued consumption in PD with a mean intake of approximately 200 mg/day among early in the disease (Schwarzschild et al., 2003a; Simon et al., 2008), its use is important to consider in A_{2A} antagonist trials. As caffeine use tends to decrease over the course of the disease, controlling for its use in the analysis of early PD/monotherapy trials may be particularly informative. If specific adenosine A_{2A} receptor antagonists were found to be more effective amongst those consuming less caffeine (i.e., less of a general adenosine antagonist), it would support the distinct possibility of a shared antiparkinsonian effect through a common mechanism. It would then remain to be determined whether specific A_{2A} receptor antagonism offers an advantage of greater efficacy or tolerability (given possibly adverse effects of blocking other adenosine receptors) to offset the lower cost and greater availability of caffeine.

Trials for disease-modification in PD

Convergent epidemiological and laboratory studies have suggested that adenosine A_{2A} antagonism may confer disease modifying benefits beyond the antiparkinsonian motor effects now being actively pursued in clinical trials as above (Schwarzschild et al., 2006; see Figure 3). Although caffeine itself was listed amongst the most attractive candidate neuroprotectants under consideration for clinical investigation (Ravina et al., 2003), if a specific A_{2A} antagonist were to receive an indication for symptomatic relief it may be more likely than caffeine to first undergo testing in a ‘neuroprotection’ trial given the high costs of conducting the necessarily large and long-term trials required.

The prospects of favorable disease modification by A_{2A} receptor blockade may extend beyond its neuroprotective potential as A_{2A} receptors have also been implicated in the maladaptive neuroplasticity that underlies the development of levodopa-induced dyskinesia (LID) in PD (Morelli et al., 2009). Accordingly, early treatment with an A_{2A} antagonist *versus* placebo as an adjunct to newly initiated levodopa may also be considered as a novel trial design to assess for both an early synergistic benefit (possibly with greater sensitivity than a monotherapy design), as well as for a prophylactic effect on LID during a second long-term phase of observation with subjects maintained in their blinded treatment arms.

6) Urate as a novel target for neuroprotection

Biology

Evolutionary significance—During primate evolution a series of mutations occurred in the urate oxidase gene (*UOx*) likely accounting for the relatively high levels of urate (the physiologically dissociated form of uric acid) in apes and humans compared to other mammals (Oda et al., 2002; see Figure 3). Urate is not only the end product of the metabolism of purines like adenosine. It also possesses antioxidant properties comparable to those of ascorbate (Ames et al., 1981) and accounts for most of the antioxidant capacity in

human plasma (Yeum et al., 2004), supporting the hypothesis that our ancestors gained antioxidant benefits from *UOx* mutations and a resulting elevation of urate concentrations (Proctor, 1970). While higher levels of urate may have conferred an evolutionary advantage through bolstered defenses against oxidative damage (Ames et al., 1981) today they are also the core molecular culprit in gout and uric acid kidney stones.

Neuroprotective effects in cellular models of PD—In addition to its antioxidant actions, urate has also been shown to possess other potentially protective properties *in vitro*. It has been shown to scavenge peroxynitrite as well as oxygen free radicals *in vitro* (Whiteman et al., 2002; Franzoni et al., 2006) and displays potent iron-chelating activity independent of its direct antioxidant action (Davies et al., 1986). Direct evidence for a neuroprotective effect of urate has come initially from cellular and animal models of multiple sclerosis, (Hooper et al., 1997, 1998; Scott et al., 2002) stroke (Yu et al., 1998; Romanos et al., 2007) and spinal cord injury (Scott et al., 2002; Du et al., 2007).

Urate also confers protection in cellular models of PD. In PC12 cells, urate blocked apoptosis and oxidant production induced by dopamine (Jones et al., 2000) or the pesticide rotenone in combination with homocysteine (Duan et al., 2002) and reduced cell death induced by MPP+ or Fe^{2+} (Haberman et al., 2007). Urate also attenuated toxin-induced loss of primary neurons in culture. A recent study of dopaminergic neurons in primary midbrain culture of rat ventral mesencephalon found that their physiological function and survival were significantly enhanced by urate, (Guerriero et al., 2009) at concentrations (≥ 30-50 μM) corresponding to those in human CSF associated with a reduced of clinical decline in PD (Ascherio et al., 2009).

Epidemiology

When first considered in case-control studies, lower urate levels were found in serum (Larumbe et al., 2001; Annanmaki et al., 2007; Bogdanov et al., 2008; Johansen et al., 2009; Andreadou et al., 2009), possibly in cerebrospinal fluid (Tohgi et al, 1993), and in post-mortem nigrostriatal tissue samples (Church & Ward, 1994) of PD patients compared to those of controls. These studies suggested that low CNS as well as peripheral levels of urate are associated with PD.

A series of epidemiological investigations of prospectively followed cohorts has more incisively linked higher blood urate with a reduced risk of developing PD (Davis et al., 1996; De Lau et al., 2005; Weisskopf et al., 2007; Chen et al., 2009). For example, in the largest of these cohorts, 18,000 men were followed for more than 8 years in the HPFS. Weisskopf and colleagues found that those in the top quartile of plasma urate concentration had a 2- to 3-fold lower risk of PD than subjects in the bottom quartile ($p<0.02$ for trend across all quartiles). Amongst the subset of cases for whom blood was collected at least four years before the diagnosis of PD, an even greater reduction of PD risk was observed – with those in the highest urate quartile having a 5-fold lower risk of PD compared to the lowest quartile ($p<0.01$ for trend). This further analysis suggests that the low uricemia among individuals with PD precedes the onset of neurological symptoms and is thus unlikely to be a consequence of changes in diet, behavior, or medical treatment early in the course of the disease. This inverse association was independent of age, smoking, caffeine consumption and other aspects of lifestyle that have been related to PD or uricemia.

Similarly, urate-elevating diet was also associated with a lower risk of PD (Gao et al., 2008). In the HPFS cohort, the authors found that a higher dietary uricemic index (reflecting dietary patterns linked to higher plasma urate) predicted a reduced risk of developing PD ($p<0.001$ for trend). The association between the index and PD risk remained strong and significant in

models further adjusted for age, smoking and caffeine intake. Their findings suggest that dietary interventions that raise blood urate concentrations might reduce the risk of PD.

In a related set of epidemiological studies of large prospectively followed cohorts, a diagnosis of gout (a form of arthritis due to urate crystallization in joints and associated with hyperuricemia) was linked to a lower risk of later being diagnosed with PD (Alonso et al., 2007; De Vera et al., 2008). Together these epidemiological data establish urate exposure – assessed by laboratory, dietary or pathological indicators – as a robust inverse risk factor for PD.

Clinical studies of urate and PD progression—The emergence of robust epidemiological data linking higher urate levels amongst healthy populations to a reduced risk of developing PD, prompted a corollary hypothesis: *Amongst people already diagnosed with PD, do higher urate levels predict a slower rate of clinical decline?* To test the hypothesis, incidentally measured urate levels in two large completed 'neuroprotection' trials were related to rates of clinical decline over years. Although neither the PRECEPT (Parkinson Study Group, 2007) nor the DATATOP (Parkinson Study Group, 1993) trial had demonstrated efficacy of candidate neuroprotectants, each had collected data for routine safety lab tests -- including serum urate -- at enrollment of some 800 recently diagnosed '*de novo*' PD patients, who were then followed closely for two years. For both trials the primary outcome was time to disability warranting the initiation of levodopa or dopaminergic agonist therapy, with secondary outcomes including rate of UPDRS change and, in the case of the PRECEPT trial, rate of loss of dopamine transporter (DAT) ligand uptake in the striatum.

In the PRECEPT cohort, subjects with higher (but still normal) levels of serum urate at baseline were significantly less likely to develop disability warranting dopaminergic therapy and also retained significantly more striatal DAT binding capacity during the study (Schwarzschild et al., 2008). For example, subjects in the top quintile of serum urate (~7-8 mg/dL with normal value reference ranges typically 3-8 mg/dL) reached the end point at only half the rate of subjects in the lowest quintile (hazard ratio, 0.51; 95% confidence interval, 0.37-0.72; p for trend <0.001). In the DATATOP cohort higher baseline urate concentrations in CSF as well as in serum were similarly associated with a slower rate of reaching the primary disability endpoint (Ascherio et al., 2009). In both cohorts higher urate levels were also predictive of a favorable rate of clinical decline measured by the change in UPDRS score. Although several descriptive clinical features of PD have been identified as probable predictors of the rate of clinical decline in PD (Post et al., 2007), urate may be the first molecular factor clearly linked to clinical progression of idiopathic PD.

In addition to its emerging utility as a prognostic biomarker of PD in research studies, the demonstrated antioxidant and neuroprotective properties of urate raise the possibility of its potential for direct therapeutic benefit. Convergence of these biological, epidemiological and clinical findings has prompted rapid translation to clinical application (Parkinson Study Group; clinical trials.gov identifier # NCT00833690). A multi-center, randomized, placebo-controlled trial of inosine (a precursor of urate as well as the deamination product of adenosine in purine metabolism; see Figure 3) in early PD is currently underway to assess its safety and ability to elevate serum and CSF urate levels, and its potential for further development as a novel strategy to impede progression of the disease.

Acknowledgments

Supported by NIH grants K24NS060991 and R01NS054978, DoD W81XWH-04-1-0881 and the RJG Foundation

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List of abbreviations

BRET	Bioluminescence Resonance Energy Transfer
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
CPX	8-cyclopentyl-1,3-dipropylxathine
CREB	cAMP response element-binding
DARPP-32	dopamine and cAMP regulated phosphoprotein-32
DAT	dopamine transporter
ERK	Extracellular signal-regulated kinases
FDA	US Food and Drug Administration
FRET	fluorescence resonance energy transfer
GABA	gamma-aminobutyric acid
GDNF	glial derived neurotrophic factor
GLT-1	glutamate transporter-1

HPFS	Health Professionals Follow-up Study
L-DOPA	L-3,4-dihydroxyphenylalanine
LID	L-DOPA induced dyskinesia
LPS	lipopolysaccharide
MAPK	mitogene-activated protein kinase
MPTP	1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine
NDA	New Drug Application
NGF	nerve growth factor
NHS	Nurses Health Study
NO	nitric oxide
3-NP	3-nitropropionic acid
6-OHDA	6-hydroxydopamine
PD	Parkinson's disease
STN	subthalamic nucleus
TH	tyrosine hydroxylase
UPDRS	Unified Parkinson's Disease Rating Scale

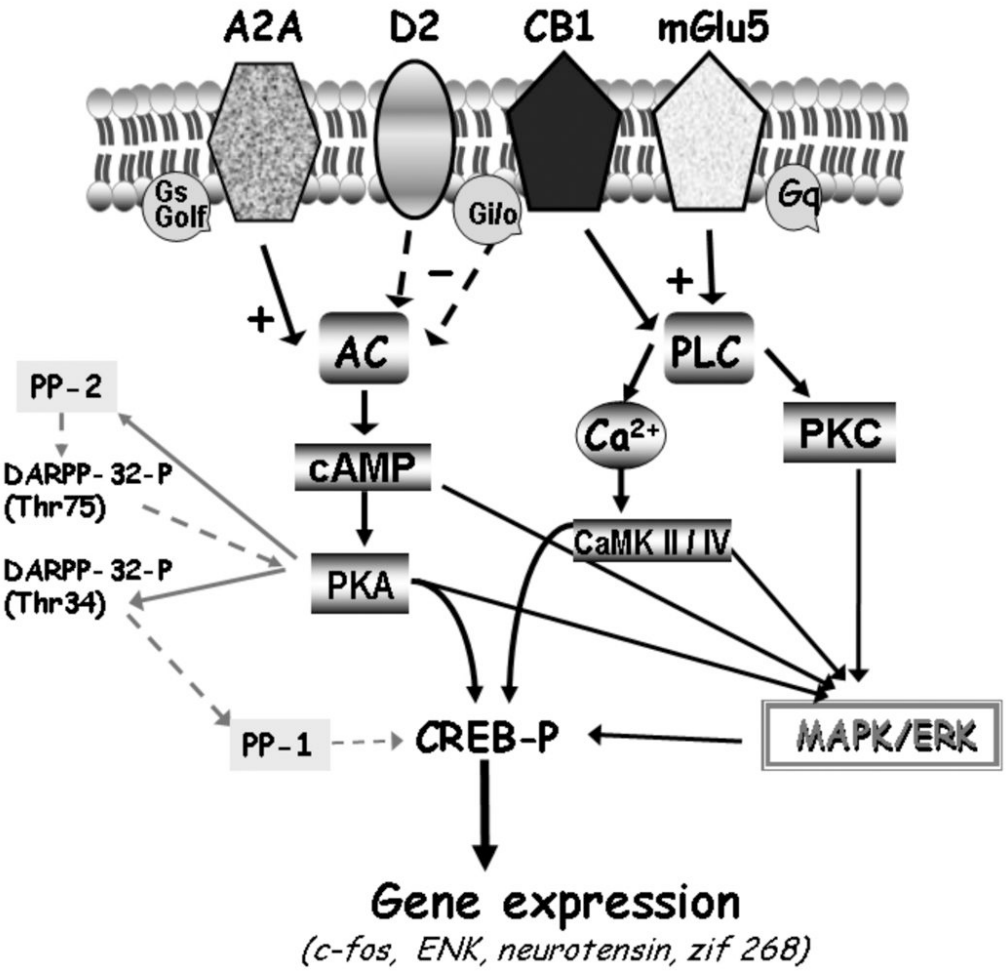

Fig 1.

Functional interactions between dopamine D_2, adenosine A_{2A}, cannabinoid CB_1 and glutamate mGlu5 receptors in striatopallidal neurons. Adenosine A_{2A} receptors interact antagonistically with D_2 and CB_1 receptors at the intramembrane level and at the adenylyl cyclase level; Metabotropic glutamate mGlu5 and adenosine A_{2A} receptors act synergistically to counteract the D_2 dopamine receptor signalling in striatopallidal neurons. Synergistic interactions exist between A_{2A} and mGlu5 receptors at the level of c-fos expression, MAP kinases and phosphorylation of DARPP-32 protein; for further explanation see text. broken arrows – inhibitory effect; '+'- stimulation; '- ' – inhibition; AC – adenylyl cyclase; Ca2+ - calcium ions; CaMK II/IV calcium/calmodulin –dependent protein kinase type II/IV; cAMP – cyclic AMP; CREB – cAMP response element-binding protein; K+ - potassium channel; DARPP-32 -dopamine and cAMP-regulated phosphoprotein; DARPP-32-P (Thr75) and DARPP-32-P (Thr34) – DARPP-32-phopshorylated at threonine residues 75 and 34, respectively; Gi, Go – inhibitory G-proteins, Gq, Gs, Golf – stimulatory G-proteins; MAPK – mitogen-activated protein kinase; PKA - protein kinase A; PKC - protein kinase C; PLC – phospholipase C; PP-1 – protein phosphatase-1 ; PP-2 - protein phosphatase-2.

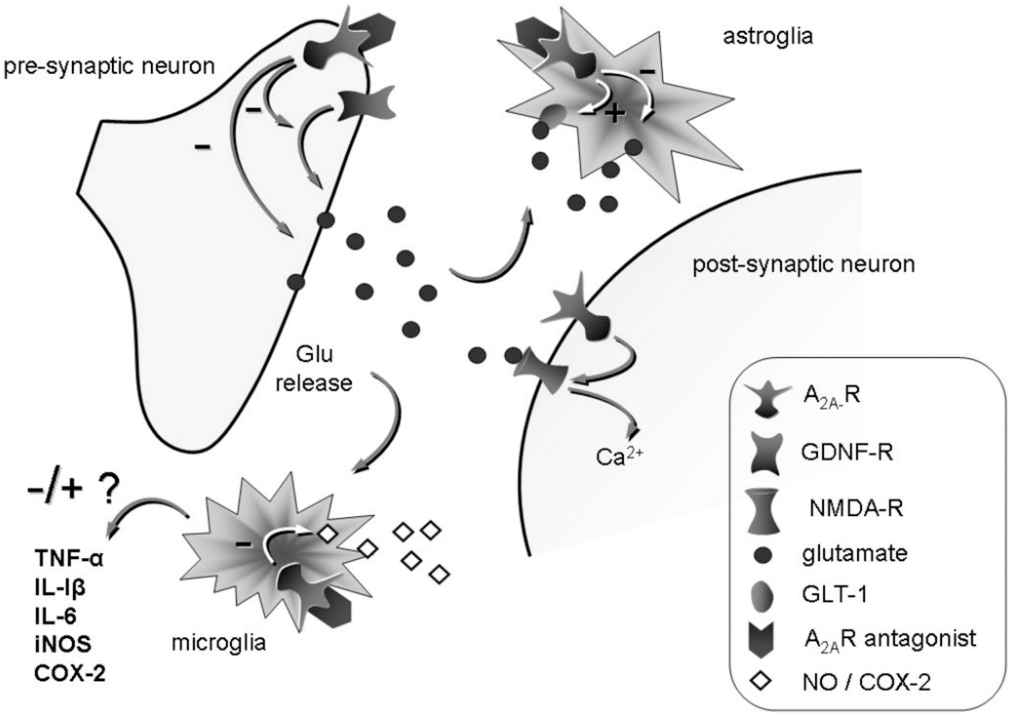

Fig. 2.

Schematic representation of possible cellular mechanisms affected by A_{2A} receptor blockade in a neuroprotective model of PD. In presynaptic neurons, A_{2A} antagonism inhibits glutamate efflux either directly or indirectly through an inhibition of GDNF receptors (see text for more details on this mechanism). A decrease in glutamate release results in reduced glial response and as a consequence release of toxic factors. In microglia, direct A_{2A} receptor blockade inhibits NO and COX-2 production. In astroglia, direct A_{2A} receptor blockade inhibits glutamate release directly or indirectly through an inhibition of GLT-1.

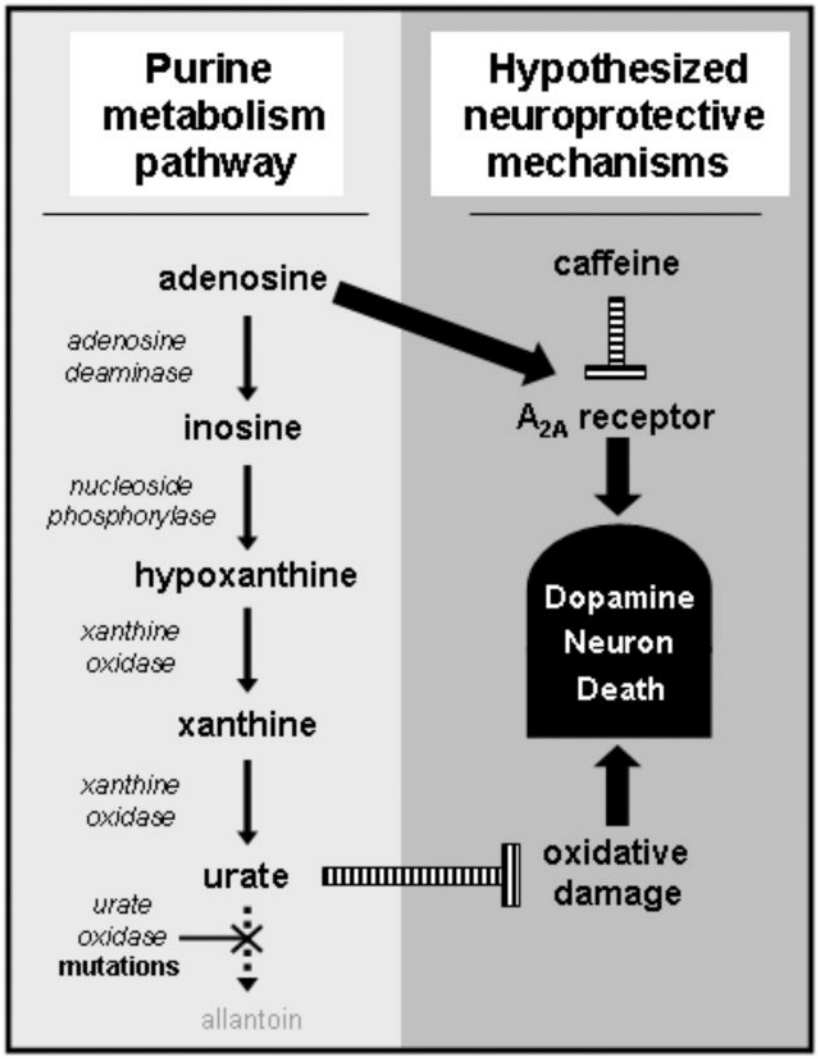

Fig 3.

Therapeutic targets along the purine metabolic pathway. Adenosine A_{2A} antagonists (including caffeine) and urate have emerged as realistic candidate neuroprotectants. In humans the enzymatic metabolism of purines such as adenosine ends with urate due to multiple mutations within the *urate oxidase* gene during primate evolution (see text). The schematic suggests a possible homeostatic mechanism linking an adenosinergic neurodegenerative influence with an offsetting neuroprotective influence of urate.

Table 1

Neuroprotective outcome of $A_{2A}R$ manipulation in different rodent PD models

Summary of recent data highlighting the neuroprotective outcome observed in both the striatum and the substantia nigra by pharmacologically and genetically targeting the A_{2A} receptor in different PD models.

PD model	A2AR manipulation	Functional Protection in Str	SNc neurons survival	Attenuation of glial response	Refs
6-OHDA in Str	A_{2A} antag.	+	+	NA	Ikeda et al, 2002
MPTP acute	A_{2A} antag.	+	+	+	Chen et al, 2001 Ikeda et al, 2002 Pierri et al, 2005 Yu et al, 2008
	totA_{2A}KO	+	NA	NA	
	fbnA_{2A}KO	-	NA	NA	
MPTP subchronic	A_{2A} antag.	+	+	+	Carta et al, 2009
	fbnA_{2A}KO	+	+	+	

Prog Brain Res. Author manuscript; available in PMC 2011 May 26.