Protective Agents in Parkinson's Disease: Caffeine and Adenosine A_{2A} Receptor Antagonists

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Abstract

Based on several findings suggesting that the adenosinergic system is one of the most interesting in the field of neuroprotection in Parkinson's disease, this chapter describes the functions of the purine adenosine and its A_{2A} receptors in the central nervous system, with emphasis on their role in neuroprotection. The neuromodulatory role of A_{2A} receptors and the preclinical and epidemiological studies on the mechanisms of the neuroprotective role of caffeine and urate, the final product of purine catabolism, are extensively discussed in the light of their potential modifying effects on Parkinson's disease.

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Keywords

6-OHDA · Animal models · Basal ganglia · Epidemiological studies · Glia · MPTP · Neurodegeneration · Neuroinflammation · Purines · Urate

Abbreviations

5-HT_{1A}	Serotonin receptors type-1A
6-OHDA	6-Hydroxydopamine
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BG	Basal ganglia
CB_1	Cannabinoid receptors type-1
CNS	Central Nervous System
CPS	Cancer Prevention Study
CPu	Caudate-putamen nucleus
CSC	8-(−3-Chlorostyryl) caffeine
DA	Dopamine
GABA	γ-Aminobutyric acid
GABAergic	γ-Aminobutyric acidergic
GPe	External portion of globus pallidus
GPi	Internal portion of globus pallidus
HD	Huntington's disease
HHP	Honolulu Heart Program
HPFS	Health Professionals Follow-Up Study
L-DOPA	L-3,4-Dihydroxyphenylalanine
$mGLU_4$	Metabotropic glutamate receptors type-4
$mGLU_5$	Metabotropic glutamate receptors type-5
MPEP	2-Methyl-6-(phenylethynyl)pyridine
MPP+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NHS	Nurses' Health Study
NIH	National Institutes of Health
NMDA	N-Methyl-D-aspartate
PD	Parkinson's disease
SNc	Substantia nigra pars compacta
SNr	Substantia nigra *pars reticulata*
STN	Subthalamic nucleus
TH	Tyrosine hydroxylase
UPDRS	Unified Parkinson's Disease Rating Scale
ZM 241385	4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol

1 Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, with an occurrence that tends to rise dramatically with aging, thereby amplifying the social relevance of the disease as life expectancy increases. The degeneration of dopaminergic neurons of the substantia nigra *pars compacta* (SNc) that project to the striatum is the hallmark of PD, and provokes functional modifications within the basal ganglia (BG) circuitry causing the typical motor symptoms (tremor, rigidity, and bradykinesia) that feature the disease. Besides being a movement disorder, PD is a complex pathology characterized by the involvement of several neuronal populations throughout the central and peripheral nervous systems. Indeed, neurotransmitters other than dopamine (DA) are involved in the disease, including noradrenaline, serotonin, and acetylcholine.

The primary cause of the degenerative process underlying PD remains unknown; however, several contributing factors as mitochondrial defects, oxidative damage, abnormal protein aggregation, and neuroinflammation have been associated to the neurodegeneration. These processes, once initiated, continue to produce a demise of dopaminergic neurons, thus contributing to the loss of efficacy of DA-replacement therapy. The principal attention in the field of PD has focused on the improvement of DA-replacement therapies, in order to better manage or prevent the onset of motor complications and, second, on the discovery of compounds that could modify the course of neurodegeneration. Among the various therapeutic approaches aimed at addressing these goals, the manipulation of adenosine A_{2A} receptors is one of the most valuable.

The suggestion that manipulating adenosine A_{2A} receptors, and adenosinergic transmission in general, might have a beneficial effect on PD onset and progression comes from three major lines of evidence: (1) epidemiological studies showing that consumption of caffeine, a nonselective A_1/A_{2A} receptor antagonist, was associated with reduced risk of developing PD; (2) preclinical studies demonstrating that the adenosine receptor type involved in these effects is the A_{2A} receptor; and (3) epidemiological studies showing that increased plasma levels of urate (the final product of purine catabolism) may be associated with reduced risk of developing PD and to a slower progression of the disease.

2 Neuromodulatory Role of Adenosine

Adenosine is an endogenous purine nucleoside constitutively present in mammalian tissues, where it plays a regulatory role in a variety of important physiological processes by acting as a homeostatic modulator. Adenosine originates from the hydrolysis of adenosine monophosphate (AMP) operated by ecto-5′-nucleotidase; thus, its formation depends upon the metabolism of adenosine triphosphate (ATP). In the extracellular compartment, the levels of adenosine also depend on the rate of

hydrolysis of ATP, which is released from either neurons or glial cells, and on the function of the adenosine carrier, which keeps adenosine at a stable level. The actions of adenosine are mediated by specific G-protein-coupled receptors, and to date four adenosine receptors have been cloned and characterized, namely, A_1, A_{2A}, A_{2B}, and A_3.

Although all the four adenosine receptor subtypes are present in the Central Nervous System (CNS), the central effects of adenosine are mainly mediated by the A_1 receptors, which are predominantly expressed in the cortex, cerebellum, hippocampus, autonomic nerve terminals, spinal cord, glial cells, and by the A_{2A} receptors, which are widely present in the striatum, olfactory tubercle, astrocytes, microglia, and oligodendrocytes (Borea et al., 2018). Adenosine modulates several physiological and pathological phenomena in the CNS. For example, adenosine regulates the sleep-wake cycle and is a critical player in the generation of long-term memories (Borea et al., 2018). Besides, adenosine plays a role in brain disorders, and both A_1 and A_{2A} receptors seem to be involved in these effects. For example, pharmacological activation or antagonism of A_1 receptors in mice has been associated with anxiolytic or anxiogenic effects, respectively, whereas rats overexpressing A_{2A} receptors in the hippocampus, cortex, and striatum have been demonstrated to display depressive-like behavior and anhedonia (Borea et al., 2018).

The ability to afford neuroprotection is another effect of adenosine that has been widely demonstrated in experimental models of diseases of the CNS. Indeed, a marked elevation in the extracellular levels of adenosine has been found after the exposure to a diverse array of noxious stimuli that compromise energy metabolism (i.e., hypoxia, ischemia, hypoglycemia, or aglycemia), eventually leading to reduced damage of the neuronal tissue (Borea et al., 2018). Neuroprotection by adenosine has also been demonstrated against neuronal damage not related to an impairment of energy metabolism, such as mechanical cell injury and neuroinflammation/neurotoxicity induced by amphetamine-like psychostimulant drugs. In general, the neuroprotective effects of adenosine appear to be mediated primarily via A_1 receptor activation, whereas the activation of A_{2A} receptors may result in neurotoxic effects. In this regard, it is noteworthy that the activation of A_1 receptors suppresses excitatory transmission by inhibiting N-type calcium-channels and inducing neuronal hyperpolarization, eventually reducing glutamate release and affording prevention/protection against neuronal damage. Conversely, increased A_{2A} receptor function bolsters the release of glutamate and the activation of N-methyl-D-aspartate (NMDA) glutamate receptors, eventually leading to synaptotoxicity (Borea et al., 2018).

The hypothesis that the activation of A_{2A} receptors may exacerbate neuronal damage is of relevance to PD, as indicated by several pharmacological studies. Thus, both the non-selective A_1/A_{2A} receptor antagonist caffeine and/or selective antagonists of A_{2A} receptors have been found to afford neuroprotection, mitigate glial cell activation, and improve behavioral deficits in animal models of dopaminergic neurodegeneration induced by toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and rotenone (see Pinna et al., 2018).

Finally, the adenosinergic modulatory system in the brain undergoes modifications with aging. Different studies have indeed evidenced a decreased density and efficiency of inhibitory A_1 receptors both in aged rodents (i.e., mice and rats) and elderly humans, as well as increased density and efficiency of A_{2A} receptors in aged rats; these changes in adenosine receptors are thought to contribute to the increased susceptibility to brain diseases that occurs in the elderly (Borea et al., 2018).

3 Adenosine A_{2A} Receptors

Among the different adenosine receptors, the A_{2A} receptors are of particular relevance for PD (see Pinna et al., 2018) since, as mentioned in Sect. 2, they are highly expressed in the dorsal and ventral portions of the striatum, the brain region that is innervated by the mesencephalic dopaminergic neurons that degenerate in the course of PD. Within the striatum, adenosine A_{2A} receptors are prevalently expressed in the soma, dendrites, and dendritic spines of the striatal γ-aminobutyric acidergic (GABAergic) neurons of the striatopallidal (indirect) pathway, where they colocalize with DA D_2 receptors. In contrast, very few striatal GABAergic neurons of the striatonigral (direct) pathway that express DA D_1 receptors also express A_{2A} receptors (Fig. 1). Nevertheless, activation or blockade of A_{2A} receptors in the indirect pathway can either impair or facilitate the responses mediated by dopaminergic D_1 receptors, by means of indirect mechanisms within the BG loop. In addition to being expressed in striatal GABAergic neurons, A_{2A} receptors are present in glial cells. Interestingly, the expression of A_{2A} receptors has been found to be very low in microglia and astrocytes under physiological conditions, but to increase dramatically in response to brain insults. In astrocytes, A_{2A} receptor stimulation promotes cell proliferation, inhibits the expression of inducible nitric oxide synthase, and increases the release of glutamate. In microglia cells, A_{2A} receptor stimulation increases cellular activation and facilitates the release of cytokines.

Adenosine A_{2A} receptors have been described to interact, either directly or indirectly, with several receptors, and these interactions are a major mechanism that underlies the neuromodulatory effects of adenosine (see Pinna et al., 2018). In this connection, the best example is the allosteric antagonistic receptor-receptor interaction between A_{2A} and DA D_2 receptors, by means of which the activation of A_{2A} receptors reduces the affinity of orthosteric ligands for the D_2 receptor binding sites and lowers G-protein coupling, thus attenuating the downstream signal transduction. Interestingly, A_{2A}-D_2 receptor-receptor interactions have been reported at multiple levels in the striatal network; for instance, on somatodentritic striatopallidal neurons, on corticostriatal glutamatergic terminals, as well as on cholinergic interneurons. Moreover, A_{2A} receptors negatively interact with dopaminergic D_3 receptors at the level of the ventral striatum, by reducing the affinity of DA for the high-affinity binding site on D_3 receptors. In addition to interacting with dopaminergic receptors, A_{2A} receptors may physically and functionally interact with glutamatergic, cannabinoid, and serotonergic receptors, as well as with adenosinergic receptors of the A_1 subtype (Fig. 1). For instance, at the level of striatal glutamatergic terminals, A_{2A} receptors finely regulate

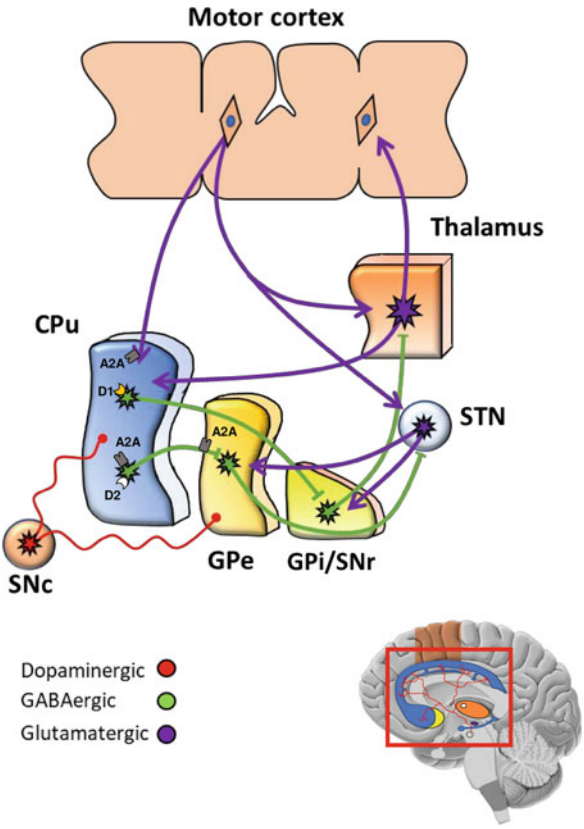

Fig. 1 Graphical illustration of A_{2A} receptors distribution within the basal ganglia (BG) circuitry. Glutamatergic neurons originating from the primary motor cortex, supplementary motor area, cingulate motor cortex, and premotor cortex form excitatory synapses projecting to the caudate-putamen nucleus (CPu), the thalamus, and the subthalamic nucleus (STN). In the CPu, sensory-motor information arriving from cortical areas are elaborated in GABAergic medium-sized spiny neurons and refined by dopaminergic inputs originating from the substantia nigra pars compacta (SNc). Conventionally, medium-sized spiny neurons are classified in two subgroups, namely, striatonigral and striatopallidal, which differ in their projection sites and in the selective expression of DA D_1 and D_2 receptors. Striatonigral medium-sized spiny neurons selectively express D_1 receptors and constitute the direct pathway by projecting to the canonical BG output nuclei, the internal portion of globus pallidus (GPi), and the substantia nigra pars reticulata (SNr). Conversely, striatopallidal medium-sized spiny neurons express high levels of D_2 and A_{2A} receptors and form the indirect pathway by projecting to the external portion of globus pallidus (GPe) that in turn makes inhibitory connections with the STN. The STN is mainly composed of glutamatergic neurons, which project to the GPe and GPi/SNr, where they form excitatory synapses onto GABAergic output neurons. Eventually, GABAergic output neurons of the BG and SNr inhibit the activity of excitatory ventroposterior thalamic motor nuclei, which in turn close the circuit by projecting back to the cortex. Adenosine A_{2A} receptors located in corticostriatal terminals as well as on striatopallidal neurons critically modulate the activity of the indirect pathway, and in turn of the BG circuitry. Legend: red lines indicate dopaminergic projections; green lines indicate GABAergic projections; purple lines indicate glutamatergic projections. The red square on the bottom indicates the anatomical location of BG structures within the human brain

the release of glutamate into the synaptic cleft by negatively interacting with both adenosine A_1 receptors and metabotropic glutamate receptors type 4 ($mGLU_4$).

Furthermore, A_{2A} receptors located on GABAergic striatopallidal neurons positively interact with both metabotropic glutamate receptors type 5 ($mGLU_5$) and cannabinoid receptors type 1 (CB_1) (see Pinna et al., 2018). Specifically, the combined activation of A_{2A} and $mGLU_5$ receptors synergistically counteracts the inhibition of striatopallidal neurons mediated by the activation of DA D_2 receptors, in turn promoting pallidal γ-aminobutyric acid (GABA) release. Conversely, the stimulation of A_{2A} receptors, in the context of A_{2A}-CB_1 heteromers, has been demonstrated as a critical mechanism that mediates the motor-depressant effects of endocannabinoids (Fig. 2). Other receptor-receptor interactions involving the A_{2A}, NMDA, and serotonin receptors type 1A ($5\text{-}HT_{1A}$) have also been reported, although further investigations are required to better define the biological significance of these interactions. Owing to these multiple receptor-receptor interactions, novel potent and selective ligands which dually act on A_{2A} receptors and on other receptors or enzymes are being designed to provide improved therapeutic effects in PD.

4 Pharmacological Properties of Caffeine

Caffeine is a xanthine alkaloid that is contained in plants used for dietary purposes (i.e., coffee, tea, cocoa beans, yerba mate leaves, guarana berries). Caffeine can also be found as an additive in foods and beverages (i.e., soft drinks, energy drinks, and energy shots), or as a component of over-the-counter medications and products marketed to increase alertness and reduce fatigue. Caffeine elicits psychostimulatory effects that are qualitatively similar to those of classical psychostimulants, though they are usually of milder intensity and not accompanied by harmful unwanted consequences. Moreover, caffeine may increase alertness and reduce mental and physical fatigue. All together, these effects render caffeine the most consumed psychoactive substance worldwide.

At doses in the range of those featuring recreational consumption, caffeine induces its central effects by blocking the adenosine A_1 and A_{2A} receptors (Ferré et al., 2018). Although caffeine does not directly bind to DA receptors, it potentiates both D_1 and D_2 receptor-mediated behavioral responses, which can be explained based on both the synaptic localization of A_{2A} receptors and the negative interactions between A_{2A} and DA receptors (see Sect. 3). In this regard, studies in experimental rodents have demonstrated that the combined administration of caffeine with either dopaminergic agonists or dopaminergic psychostimulants leads to a more marked behavioral activation, compared with the administration of either drug alone (Tronci et al., 2006). Moreover, rodents repeatedly exposed to caffeine may display an increased responsiveness to the behavioral and neurochemical effects of the dopaminergic psychostimulant amphetamine that persists even after caffeine discontinuation (Tronci et al., 2006). On the other hand, an attenuation in the psychostimulant effects of caffeine can be observed after the administration of DA receptor antagonists (Tronci et al., 2006). The evidence that caffeine may influence dopaminergic

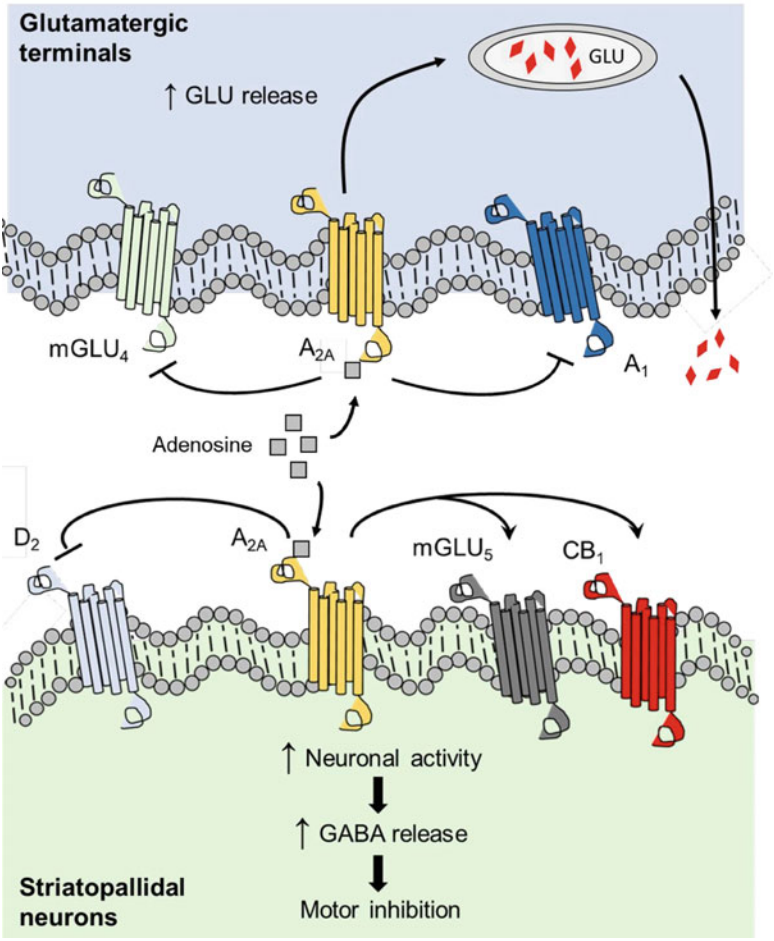

Fig. 2 Schematic representation of A_{2A} receptor functions and interactions at the level of the indirect striatal pathway. At the presynaptic level, A_{2A} receptors finely regulate the release of glutamate from corticostriatal terminals. For instance, activated A_{2A} receptors negatively interact with glutamatergic $mGLU_4$ and adenosine A_1 receptors, eventually enhancing the presynaptic release of glutamate. At the postsynaptic level, activated A_{2A} receptors positively interact with glutamatergic $mGLU_5$ and cannabinoid CB_1 receptors and counteract the inhibition of GABAergic striatopallidal neurons mediated by DA D_2 receptors, thus increasing the overall firing activity of the indirect striatal pathway. *GLU,* glutamate

transmission is of great interest, considering the critical involvement of DA in the modulation of major brain functions, such as movement, attention, goal-directed behavior, and associative learning. Moreover, caffeine is one of the dietary factors whose consumption has been correlated with reduced incidence of PD, which acquires particular interest in light of the fact that caffeine is generally considered a safe substance and is extensively consumed worldwide (see Sect. 5). In this regard,

the results of preclinical studies demonstrating that caffeine can afford protective effects in different models of neurotoxicity add further importance to the findings of epidemiological studies linking caffeine to PD (see Sects. 5 and 6). Thus, caffeine administration has been shown to elicit neuroprotective effects in rodent and primate models of PD-like dopaminergic neuron degeneration, such as that induced by either MPTP or 6-OHDA (see Sect. 6). Neuroprotective properties of caffeine are particularly important, since they can be afforded by a substance that is already available in the market and elicits negligible toxic or adverse effects and that could, in turn, represent a readily available therapeutic option.

5 Epidemiological Studies on the Neuroprotective Role of Caffeine in Parkinson's Disease

Epidemiological studies investigating habits, health status, and lifestyles of individuals who developed PD are very important in order to obtain new information about the factors that promote disease development, and to identify new approaches to prevent the neurodegeneration underlying PD.

One of the most interesting results that have emerged from these epidemiological studies is the inverse correlation between the intake of caffeine and the risk of developing PD. The major epidemiological studies that provided consistent suggestions regarding the existence of such an inverse correlation are the Honolulu Heart Program (HHP, 30 years of follow-up of ~8000 Japanese-American men), the Health Professionals Follow-Up Study (HPFS, 10 years of follow-up of ~47,000 men), the Nurses' Health Study (NHS, 16 years of follow-up of ~88,500 women), the National Institutes of Health-AARP Diet and Health Study (NIH-AARP Diet and Health Study, ~10 years of follow-up of ~318,260 American participants, of which 187,499 were men and 130,761 women), and the Cancer Prevention Study II Nutrition Cohort (CPS II-Nutrition, ~8 years of follow-up of ~184,190 American participants, of which 86,404 were men and 97,786 women) (Liu et al., 2012; Palacios et al., 2012; Ren and Chen, 2020).

In these studies, surveys were conducted to collect information about lifestyles, health status, and nutritional behaviors of individuals, including data about the consumption of coffee and/or other caffeinated/decaffeinated beverages. The results of these investigations consistently demonstrated that individuals who consumed medium (three cups of coffee/day) to high (five cups or more/day, including tea and other caffeine sources) amounts of caffeine had a decreased risk of developing PD (Liu et al., 2012; Palacios et al., 2012; Ren and Chen, 2020). Importantly, these effects were found to be dose-dependent, and were adjusted for age, smoking status, alcohol use, intake of nutrients other than caffeine contained in coffee, and potential confounding variables (i.e., saturated fat level, physical activity, total energy intake) (Liu et al., 2012). Overall, epidemiological studies indicated that individuals who regularly consumed caffeine had a lower risk of developing PD compared with non-regular caffeine drinkers (i.e., people who consumed less than one cup a day), whereas no modifications in the incidence of

PD were found in people who consumed decaffeinated coffee. Moreover, a reduction of 50% in the risk of developing PD was observed among men who consumed less than one cup of coffee per day compared with men who did not consume coffee at all (Ascherio et al., 2003).

Notably, a few studies demonstrated that the inverse association between caffeine intake and risk of developing PD was less evident in women than in men (Ascherio et al., 2003; Palacios et al., 2012). It was hypothesized that such a gender-dependence of effect could stem, at least in part, from the difference in sexual hormones between men and women; accordingly, a further analysis was performed in the women enrolled in the NHS and CPS II-Nutrition studies, to ascertain the correlation among use of postmenopausal estrogens, caffeine intake, and risk of developing PD (Ascherio et al., 2003; Palacios et al., 2012). Generally, the use of postmenopausal estrogens was found not to be correlated with the risk of developing PD. Nevertheless, analysis narrowed to estrogen users showed that women who consumed more than five cups of coffee per day had a higher risk of developing PD, compared with women who never drank coffee (Ascherio et al., 2003); conversely, use of estrogens was associated with a reduced risk of developing PD among those women who showed low caffeine consumption (Ascherio et al., 2003). In addition, and similar to men, women who were coffee drinkers and had never taken postmenopausal estrogens had a lower risk of developing PD than women who did not consume coffee at all (Ascherio et al., 2003; Palacios et al., 2012). These data were confirmed by different prospective studies involving men and women, which provided evidence suggesting that caffeine could decrease the risk of developing PD in men as well as in women who did not take postmenopausal estrogens, but not in women who were under hormonal replacement therapy (Ascherio et al., 2003; Palacios et al., 2012). However, these findings have not been replicated by other prospective studies in men and women, which reported that the inverse association between coffee intake and risk of developing PD was not influenced by gender (Liu et al., 2012). Nevertheless, subsequent systematic reviews and meta-analysis studies, which included epidemiological and case control-studies, have unequivocally demonstrated the existence of an inverse association between caffeine intake and reduced risk of developing PD, also confirming that such an inverse association is stronger in men than in women (Hong et al., 2020).

An experimental study in the MPTP mouse model of PD has investigated the biological basis of the interaction between estrogen hormones and caffeine, and how such an interaction may affect the degeneration of the nigrostriatal dopaminergic system. That study showed that caffeine, dose-dependently, attenuated the loss in striatal DA induced by MPTP in both male and ovariectomized female mice, compared with non-ovariectomized female mice (Xu et al., 2006). Moreover, chronic treatment with estrogens counteracted the neuroprotective effects that caffeine elicited in both male and ovariectomized female mice treated with MPTP, confirming that hormonal therapy might prevent the neuroprotection mediated by caffeine in this model of PD (Xu et al., 2006). The existence of metabolic and

pharmacokinetic interactions between caffeine and estrogens has been proposed (i.e., competitive inhibition of P450 by caffeine and estrogen), but it is not clear whether these interactions underlie the reduction in the neuroprotective effects of caffeine that are observed when this psychostimulant is combined with estrogen therapy (Xu et al., 2006). Overall, the contradictory results regarding the influence of estrogens treatment on PD development in women may be due to several causes, and among them could be of importance the presence of differences in estrogen levels, type of hormones used, duration of hormone use, and age. Moreover, the amount of caffeine consumed may as well be a factor to be considered in this respect (Ascherio et al., 2003; Palacios et al., 2012). Therefore, the mechanisms underlying the relationship among estrogen use, caffeine intake, and modified risk of developing PD are still ill defined; elucidating this issue requires more careful and extensive investigations with a specific design that considers the possible differences in estrogen use in women under hormonal replacement therapy.

Even though epidemiological studies and experimental findings strongly support the concept that dietary caffeine decreases the possibility of developing PD, very little is known about the association between caffeine intake and disease progression in patients already diagnosed with PD. Three clinical studies have failed to provide clear evidence of a beneficial effect of dietary caffeine on the progression of PD evaluated by means of the modified Unified Parkinson's Disease Rating Scale (UPDRS) (Simon et al., 2015). In particular, the largest study conducted in this regard showed no association between caffeine intake and PD progression, except in patients taking creatine who displayed an accelerated disease progression (Simon et al., 2015). These data indicate a potentially deleterious interaction between caffeine and creatine with respect to the rate of PD progression, although it is noteworthy that this study was neither randomized nor blinded with respect to caffeine intake (Simon et al., 2015).

Interestingly, a few clinical investigations and a meta-analysis study have reported that consumption of high amounts of caffeine (more than 200 mg/day) was associated with a reduced risk of developing dyskinesia in PD patients treated with L-3,4-dihydroxy phenylalanine (L-DOPA), and was also associated with a lower rate of starting L-DOPA treatment in de novo PD patients (Moccia et al., 2016; Hong et al., 2020). Specifically, in a population of de novo PD patients, consumption of high amounts of caffeine (more than 270 mg/day) was associated with a reduced accrual of both motor and non-motor disability and with a decreased need for L-DOPA treatment (Moccia et al., 2016). Moreover, few restricted clinical studies in PD patients demonstrated that treatment with caffeine (100–400 mg/day) had symptomatic beneficial effects on freezing of gait, motor manifestations (i.e., bradykinesia, rigidity), sleep quality, and measures of daytime sleepiness (Ren & Chen, 2020). However, another recent clinical study by Postuma and collaborators (2017) failed to reveal any beneficial effects of caffeine consumption on motor deficits in PD patients. Therefore, further larger investigations are required to thoroughly assess the neuroprotective and symptomatic effects of caffeine on disease progression in PD patients.

6 Mechanisms of Dopaminergic Neuroprotection by Caffeine and A_{2A} Receptor Antagonists

Studies of neuroprotection in models of PD should carefully consider the features of neurodegeneration (i.e., neurotoxins employed, progression, severity), as well as the experimental paradigms (i.e., *in vitro* or *in vivo*) in which the investigations are performed. In recent years, PD has been recognized as a multifactorial pathology; therefore, it is hypothesized that drugs with neuroprotective effects should act through multiple mechanisms. Moreover, it is worth mentioning that most of the experimental models of PD in use employ toxins according to an acute or acute-repeated protocol of administration (Table 1). This may lead to conditions that significantly differ from the features of idiopathic PD with respect to the time-course of degeneration, which is usually more extended in PD than in experimental models, and the mechanisms involved in neuronal demise.

Several studies in rodent models of PD have demonstrated that A_{2A} receptor antagonists counteract both the demise of dopaminergic neurons in the SNc and the drop of DA levels in the striatum (see Pinna et al., 2018). Nevertheless, A_{2A} receptor antagonists exert beneficial effects not only in models of PD-like neurodegeneration, but also in paradigms of Alzheimer's disease (AD)- and Huntington's disease (HD)-like neurotoxicity. This evidence has suggested that A_{2A} receptor antagonists counteract PD-like nigrostriatal neurodegeneration by means of mechanisms that are not selective toward dopaminergic neurons, but are part of a broader neuroprotective action. Considering these observations, two major hypotheses have been proposed to explain the neuroprotective effects of A_{2A} antagonists on dopaminergic neurons: (1) modulation of glutamate-induced excitotoxicity and (2) counteraction of neuroinflammation. Interestingly, recent evidence raised the possibility that A_{2A} receptor antagonists may also decrease alpha-synuclein aggregation at the later stages of the aggregation process, thus aiding neuroprotection in PD (Ferreira et al., 2017). Therefore, it is conceivable that an array of mechanisms may participate in the rescue of dopaminergic transmission by A_{2A} antagonists.

Glutamate-mediated excitotoxicity has long been envisaged as a crucial player in the neurodegeneration of dopaminergic neurons that underlies PD. Remarkably, the stimulation of A_{2A} receptors can elevate the extracellular concentrations of glutamate in the striatum, while A_{2A} receptor blockade exerts an opposite effect and may thus relieve the excitotoxic insult (Gołembiowska & Dziubina, 2012). Further, the selective A_{2A} receptor antagonists CSC (8-(−3-chlorostyryl) caffeine) and ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino] ethyl)phenol) in combination with L-DOPA have been found to restore striatal DA-glutamate balance and to further mitigate the overproduction of free radicals induced by 6-OHDA, which could be a mechanism involved in the neuroprotective effects of A_{2A} receptor blockade (Gołembiowska & Dziubina, 2012).

Additional studies have confirmed that the interaction between A_{2A} receptors and glutamatergic transmission may be a mechanism that regulates the survival of nigrostriatal dopaminergic neurons. Thus, the combined administration of different A_{2A} receptor antagonists with the $mGluR_5$ antagonist MPEP

Table 1 The table describes some of the toxin-based experimental models that are most commonly used to investigate at the preclinical level the degeneration of the dopaminergic nigrostriatal system, the hallmark of Parkinson's disease. The main characteristics of each model are reported, together with the effects of caffeine on the degenerative process, when available

Model	Type of treatment	Toxic effects observed	Main features of the model	Effect of caffeine administered before or concomitantly to the toxin
Cell cultures	• Acute • They may use different cells (i.e., neuroblastoma cells, mesencephalic cells) and different toxins (i.e., 6-OHDA, MPP+, rotenone)	• Decrease in cell viability	• Allows to rapidly identify putative neuroprotective agents and to study the molecular mechanisms underlying neuroprotection • Can be used as a complement of *in vivo* models	• Cytoprotection[a, b]
6-OHDA in rats	• Acute • Employs the direct intracerebral infusion of the toxin	• Degeneration of DA neurons in the SNc • Loss of DA terminals in the striatum • Reduction in the striatal levels of DA and its metabolites	• The degeneration is rapid and reaches a near terminal stage few days after toxin infusion • Oxidative stress is the major mechanism of neurotoxicity • May allow to selectively target different brain regions according to the site of toxin infusion	• Attenuation of the demise of dopaminergic neurons in the SNc[c] • Restoration of the levels of DA and its metabolites in the striatum[c]
Lipopolysaccharide in rats and mice	• Acute or chronic • Can use either intracerebral infusion or systemic administration	• Degeneration of DA neurons in the SNc • Activation of glial cells • Elevation in the levels of neuroinflammatory mediators	• Induces a selective loss of DA neurons in the SNc, while sparing other DA neurons • Degeneration of DA neurons is delayed, progressive, and irreversible • Neuroinflammation is the main mechanism underlying neuronal death	• Attenuation of neuroinflammation[d]

(continued)

Table 1 (continued)

Model	Type of treatment	Toxic effects observed	Main features of the model	Effect of caffeine administered before or concomitantly to the toxin
Paraquat or Paraquat + maneb in mice	• Chronic	• Degeneration of DA neurons in the SNc • Loss of DA terminals in the striatum	• This model may yield variable results and high rates of mortality • The toxins used are clinically relevant, since exposure to paraquat and maneb has been suggested to cause dopaminergic neurodegeneration in humans, which may eventually result in PD	• Attenuation of the demise of dopaminergic neurons in the SNc[e]
MPTP in mice	• Acute or acute-repeated with multiple administration performed at a fixed interval • The toxin is administered systemically	• Degeneration of DA neurons in the SNc • Loss of DA terminals in the striatum • Reduction in the striatal levels of DA and its metabolites • Glial activation	• The severity of DA lesion can be modified by varying the protocol of administration • It is still questioned whether or not MPTP induces a progressive DA lesion • The model can be used to study both neurotoxicity and neuroinflammation • The toxin acts chiefly on mitochondrial function • MPTP is a clinically relevant toxin, as it can induce parkinsonism in humans	• Attenuation of the demise of dopaminergic neurons in the SNc[f] • Restoration of the levels of DA and its metabolites in the striatum[g] • Counteraction of neuroinflammation[a, g]

Rotenone in cell cultures, rats and mice	• Chronic • The toxin is administered by osmotic minipumps, parentheral injection, or intracerebral infusion	• Degeneration of DA neurons in the SNc • Loss of striatal DA terminals accompanied by reduction in striatal DA content	• Degeneration of the nigrostriatal dopaminergic system is often accompanied by other non-specific brain damages • Systemic toxicity and high mortality of animals is often associated with, or precedes, dopaminergic neurodegeneration • Use of this model may yield high variability in the results obtained • Differently from other commonly used models of PD, the chronic administration of rotenone can lead to the formation of proteinaceous inclusions in DA neurons that resemble those observed in the brain of parkinsonian patients • Rotenone is a clinically relevant toxin, since exposure to rotenone has been suggested to cause dopaminergic neurodegeneration in humans, which may eventually result in PD	• Cytoprotection[h] • Restoration of the levels of DA and norepinephrine in the striatum and midbrain[i]

Abbreviations: *6-OHDA* 6-hydroxydopamine, *DA* dopamine, *MPP+* 1-methyl-4-phenylpyridinium, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *PD* Parkinson's disease, *SNc* substantia nigra *pars compacta*

[a]Lee et al., 2013; [b]Nobre et al., 2010; [c]Machado-Filho et al., 2014; [d]Badshah et al., 2019; [e]Yadav et al., 2012; [f]Xu et al., 2016; [g]Carta et al., 2009; [h]Liu et al., 2018; [i]Khadrawy et al., 2017

(2-methyl-6-(phenylethynyl)pyridine) has been found to be more effective in rescuing neuronal loss and impaired motor function in the rat 6-OHDA model of PD and in parkinsonian mice, as compared with the administration of either drug alone (Fuzzati-Armentero et al., 2015). Moreover, either lesion of the subthalamic nucleus (STN) or blockade of glutamate NMDA receptors in the STN attenuate the loss of dopaminergic neurons induced by 6-OHDA in rats (Carvalho & Nikkhah, 2001). Based on these considerations, the release of glutamate from the STN can be envisioned as an important mechanism that promotes the degeneration of dopaminergic nigral neurons. Therefore, it is conceivable that A_{2A} receptor antagonists, by regulating the activity of the striatopallidal pathway, besides counteracting motor disability, may also attenuate the degeneration of dopaminergic nigral neurons through the modulation of STN firing (Fig. 1). Finally, it is noteworthy that the release of glutamate can also be regulated by non-neuronal cells (i.e., microglia and astrocytes) where A_{2A} receptors are expressed (see Sects. 2 and 3).

Another important mechanism that is thought to participate in the neurodegenerative process underlying PD is neuroinflammation, as suggested by preclinical and epidemiological evidence (Halliday & Stevens, 2011). Neuroinflammation gives rise to a complex cascade of events in the brain, resulting in the activation of glial cells (astrocytes, microglia, and oligodendrocytes) and the subsequent release of inflammatory mediators (i.e., cytokines, interferons, interleukins) (Halliday & Stevens, 2011). Even though transient neuroinflammation can be beneficial, as it may help the brain to cope with noxious insults, protracted neuroinflammation is detrimental and may eventually lead to neuronal damage (Halliday & Stevens, 2011). It has been suggested that glia activation plays an important role in the initiation of early tissue pathological changes and in the progression of PD pathology, in which alpha-synuclein accumulation in astrocytes causes the recruitment of phagocytic microglia that target selected neurons which eventually degenerate (Halliday & Stevens, 2011). Furthermore, not only neuroinflammation can promote neurodegeneration, but neuronal death itself can trigger inflammatory responses, which may aggravate the ongoing neuronal damage (Halliday & Stevens, 2011).

Stimulation of A_{2A} receptors promotes the recruitment of non-neuronal cells in the neuroinflammatory cascade; conversely, blockade of A_{2A} receptors suppresses the activation of astrocytes and microglia, and also contrasts the stimulation of the proinflammatory signal cascade (Armentero et al., 2011). Interestingly, this notion is supported by findings demonstrating that A_{2A} receptor antagonists rescued death of dopaminergic neurons and significantly attenuated astrogliosis and microgliosis in rodent models of PD-like neurodegeneration (see Pinna et al., 2018). Recently, A_{2A} receptor antagonists have also been shown to ameliorate transcriptional deregulation, inflammation, and astrogliosis induced by the overexpression of A_{2A} receptors in primary astrocytes (Paiva et al., 2019). Therefore, glial cells may be a crucial site at which A_{2A} antagonists promote the survival of dopaminergic neurons, perhaps by attenuating both excitotoxicity and neuroinflammation. It is noteworthy that A_{2A} receptor antagonists counteract the degeneration of dopaminergic neurons in experimental models of PD at doses lower than those required to elicit motor stimulation (Carta et al., 2009). Accordingly, it has been hypothesized that A_{2A} receptor

antagonism affords neuroprotection by means of mechanisms that primarily involve the regulation of excitotoxicity and neuroinflammation, and that are then distinct from those that mediate the beneficial effects of A_{2A} receptor antagonism on motor behavior. Furthermore, it is noteworthy that excitotoxicity and neuroinflammation have been envisaged as causative factors in neurodegenerative disorders other than PD. Therefore, an attenuation, or regulation, of these noxious phenomena by A_{2A} antagonists, besides justifying the protective effect observed in PD models, would agree with the data demonstrating that these drugs can protect neurons from a wide array of insults.

Even though increasing evidence supports the involvement of glial cells in the neuroprotective effects that A_{2A} receptor antagonists exert on dopaminergic nigrostriatal neurons, the participation of neuronal mechanisms in these effects cannot be disregarded. In this context, it is worth mentioning the results of an earlier study in mice demonstrating that the inactivation of neuronal forebrain A_{2A} receptors counteracted the degeneration of dopaminergic nigral neurons in the MPTP model of PD (Carta et al., 2009). Nevertheless, it has been recently observed that neuroprotection provided by caffeine in the MPTP mouse model of PD is at least partially independent from A_{2A} receptors in forebrain neurons and/or astrocytes (Xu et al., 2016). Accordingly, further studies are necessary to determine how the A_{2A} receptors located in neuronal and non-neuronal cells contribute to the neuroprotective effects afforded by caffeine and selective A_{2A} receptor antagonists. Finally, it has been proposed that A_{2A} receptor antagonists may elicit beneficial effects also on phenomena other than excitotoxicity and neuroinflammation which are thought to regulate neuronal survival, such as autophagy, oxidative stress, and proteolytic damage. For example, the non-selective A_{2A} receptor antagonist caffeine has recently been reported to restore the mitochondrial dysfunctions induced by MPTP in mice (Essawy et al., 2017), and to protect against alpha-synucleinopathy by promoting autophagy processes in the striatum of mice injected with alpha-synuclein fibrils (Luan et al., 2018).

Antagonism of A_{2A} receptors has been demonstrated to efficiently restore motor function without exacerbating dyskinesia in animal models of PD; moreover, the A_{2A} receptor antagonist istradefylline has received the US FDA approval in 2019 as add-on therapy against the wearing-off phenomenon associated with L-DOPA therapy in advanced PD patients. Therefore, A_{2A} antagonists remain a class of drugs that are highly relevant to PD, since they not only are effective on motor symptoms, but may also possess neuroprotective potential, which is particularly desirable given the substantial lack of disease-modifying therapies for PD.

7 Potential Neuroprotective Properties of Urate in Parkinson's Disease

In recent years, several lines of clinical evidence have indicated that high plasma levels of urate, the final product of purine catabolism, may be associated with reduced risk of developing PD, as well as with slower progression of symptoms in

already diagnosed patients (Bakshi et al., 2020). Taken together, these data would suggest that urate may attenuate the degeneration of nigral dopaminergic neurons that underlies PD, an effect that may be explained by considering that urate has marked antioxidant properties, accounting itself for most (about the 60%) of the antioxidant capacity of human plasma. Indeed, increased levels of oxidative stress are among the factors that can trigger the demise of dopaminergic mesencephalic neurons in PD, as well as the neuronal loss that features other neurodegenerative conditions.

Case-control studies have observed decreased levels of serum urate in PD patients, compared with the general population; moreover, epidemiological studies have reported the existence of an inverse association between plasma levels of urate and likelihood of developing PD (Bakshi et al., 2020). Notably, an earlier epidemiological study also reported that the inverse association between uricemia and risk of developing PD was not influenced by habits and lifestyles (i.e., caffeine intake, smoking) that have been suggested to modify either uricemia or likelihood of developing PD (Weisskopf et al., 2007); this finding could strengthen the hypothesis that urate itself can influence the survival of dopaminergic nigral neurons. Coherent with this view is also the evidence that urate may be a prognostic marker of PD and that urate supplementation may possess therapeutic value in PD patients. Thus, earlier studies have reported that high levels of urate in either the serum or the cerebrospinal fluid may be associated with a slow clinical progression of PD in diagnosed patients (Bakshi et al., 2020). Notably, the value of serum urate as a prognostic marker in PD has been confirmed in more recent studies demonstrating that low plasma urate predicted the worsening of motor and/or non-motor symptoms in patients (Sleeman et al., 2019). Regarding the proposed therapeutic value of urate as a neuroprotective agent in PD, it is noteworthy that the administration of inosine, the metabolic precursor of urate, alone or in combination with the xanthine oxidase inhibitor febuxostat, has been reported to increase the serum levels of urate and also to lead to either a trend toward delayed disease progression or a significant improvement of motor symptoms in PD patients (Watanabe et al., 2020). Although the results of clinical studies about urate and PD are intriguing, it has to be mentioned that evidence also exists to indicate that high plasma levels of urate may not always be associated with reduced likelihood of developing PD and/or with delayed progression of motor symptoms (Hasimoglu et al., 2020). Moreover, other studies have suggested that high plasma levels of urate can even be detrimental in PD, as they may be associated with accelerated disease progression, decreased cognitive functions, and worsening of L-DOPA-induced dyskinesia (Jung et al., 2020; Spencer et al., 2020). Furthermore, earlier studies have suggested that the beneficial effects of urate in PD may be gender-specific and most evident in men (Baik et al., 2020). Nevertheless, other studies have found that high serum levels of urate are associated with decreased risk of developing PD in both men and women (Bakshi et al., 2020), or with increased DA transporter uptake, evaluated by positron emission tomography, in parkinsonian women but not in parkinsonian men (Oh et al., 2020). Therefore, the precise factors that regulate the effects of urate on the survival of the dopaminergic nigrostriatal system as well as the influence of

gender on these effects must be thoroughly characterized, in order to clarify the value of urate as a prognostic marker and therapeutic in PD.

The existence of neuroprotective effects of urate hypothesized by clinical studies is consistent with the results of preclinical investigations in different experimental models of PD. Investigations *in vitro* have shown that application of urate extended the survival of PC12 cells that were exposed to either DA or toxins that are known to damage the dopaminergic nigrostriatal system (Zhu et al., 2012). Besides, uric acid has been found to extend the survival of cultured midbrain neurons that were spontaneously subjected to death (Guerreiro et al., 2009). Investigations *in vivo* have substantiated and broadened the results of *in vitro* studies, by showing that the chronic administration of uric acid improved the behavioral deficits, reduced the loss of dopaminergic nigral neurons, and attenuated the depletion of striatal DA in the 6-OHDA rat model of PD (Gong et al., 2012). Similarly, consumption of a diet that increased the plasma levels of urate was found to be associated with an attenuation in both behavioral deficits and striatal depletion of tyrosine hydroxylase (TH) in 6-OHDA-lesioned parkinsonian mice (Nakashima et al., 2019). The possibility that urate has a beneficial influence on the survival of dopaminergic nigrostriatal neurons is also supported by complementary *in vivo* evidence that was obtained in transgenic mice not expressing the enzyme urate oxidase, which is responsible for the catabolism of urate, as well as in toxin-based models of PD. Thus, mice knock out for urate oxidase have been shown to possess higher brain levels of urate and to be less susceptible to 6-OHDA-induced neurotoxicity, compared with wild-type mice (Chen et al., 2013). Moreover, an elevation in the levels of urate has been demonstrated in the dopamine-denervated striatum of unilaterally 6-OHDA-lesioned rats and in the striatum of MPTP-treated mice (De Luca et al., 2014). Interestingly, in the dopamine-denervated striatum of 6-OHDA-lesioned rats, an inverse correlation was observed between the levels of urate and DA, which led to the hypothesis that insults targeting the dopaminergic nigrostriatal system could trigger an elevation in brain urate as an endogenous compensatory mechanism to contrast neurodegeneration (De Luca et al., 2014). In agreement with this hypothesis may also be the finding that in 6-OHDA-lesioned rats a reduction in the serum levels of urate is associated with increased severity of motor deficits (Sarukhani et al., 2018).

In addition to the proposed beneficial effects of urate on the degeneration of dopaminergic nigral neurons featuring PD, evidence exists to suggest that urate may exert a broader neuroprotective effect, hence being potentially valuable also in neurological conditions other than PD. Thus, preclinical studies have demonstrated that urate may improve and/or delay the onset of neurological damage in preclinical models of disease (see, e.g., Hooper et al., 2000). Moreover, clinical studies have reported reduced levels of urate in patients diagnosed with AD, HD, Lewy body dementia, or amyotrophic lateral sclerosis (ALS) and have also observed a decelerated disease progression in HD and ALS patients who had high plasma levels of urate (Paganoni & Schwarzschild, 2017). Finally, urate is being evaluated as a novel therapeutic for the treatment of ischemic stroke (Amaro et al., 2019). As mentioned above, the neuroprotective effects of urate are thought to primarily stem from its antioxidant properties, based on the evidence that increased oxidative stress is a

pathological pathway common to diverse neurodegenerative diseases. Nevertheless, additional mechanisms have been implicated in the neuroprotective properties of urate, such as metal chelating properties and regulation of astrocyte function (Bakshi et al., 2020). A thorough elucidation of these mechanisms is warranted in order to exhaustively characterize the neuroprotective properties of urate, since clarifying this issue may have important implications for the optimization of urate-based therapeutic approaches in the management of PD and other neurodegenerative conditions.

8 Conclusion

Investigations on the purinergic system have given new impetus to the research on PD. In the absence of effective neuroprotective treatments for PD, epidemiologic studies showing that consumption of the adenosine receptor antagonist caffeine is associated with a decreased risk of developing PD became compelling. Moreover, the preclinical and clinical investigations suggesting that adenosine A_{2A} receptor antagonists may exert symptomatic beneficial effects on motor deficits and also slow down, or arrest, the progression of dopaminergic neuron degeneration are very important for identifying effective therapies for PD. Nevertheless, it is noteworthy that the discovery of neuroprotective strategies in chronic neurodegenerative diseases, such as PD, largely depends on the possibility of monitoring the progression of the neuronal loss. In this regard, evidence exists that urate, the final product of purine catabolism, not only elicits neuroprotective effects in experimental models of PD, but may also mark the progression of disease in patients. Therefore, future research on the role of the purinergic system in PD is warranted, with the aim to develop new A_{2A} receptor antagonists with increased therapeutic efficacy and to further characterize urate as a reliable biomarker of disease progression.

9 Cross-References

- ▶ 6-Hydroxydopamine Lesioning of Dopamine Neurons in Neonatal and Adult Rats Induces Age-Dependent Consequences
- ▶ Alpha-Synuclein Toxicity: An Insight on Controversial Issues
- ▶ Links Between Paraquat and Parkinson's Disease
- ▶ Mechanisms of Dopamine Oxidation and Parkinson's Disease
- ▶ MPTP: Advances from an *Evergreen* Neurotoxin
- ▶ Neurotoxicity: A Complex Multistage Process Involving Different Mechanisms
- ▶ Survey of Selective Monoaminergic Neurotoxins Targeting Dopaminergic, Noradrenergic, and Serotoninergic Neurons
- ▶ The NMDA Receptor System and Developmental Neurotoxicity

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