Review Article

Effects of Caffeine in Parkinson's Disease: From Neuroprotection to the Management of Motor and Non-Motor Symptoms

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Abstract. Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population older than 60 years. Classically, PD is considered to be a motor system disease and its diagnosis is based on the presence of a set of cardinal motor signs (rigidity, bradykinesia, rest tremor) that are consequence of a pronounced death of dopaminergic neurons in the substantia nigra pars compacta. Nowadays there is considerable evidence showing that non-dopaminergic degeneration also occurs in other brain areas which seems to be responsible for the deficits in olfactory, emotional and memory functions that precede the classical motor symptoms in PD. The present review attempts to examine results reported in epidemiological, clinical and animal studies to provide a comprehensive picture of the antiparkinsonian potential of caffeine. Convergent epidemiological and pre-clinical data suggest that caffeine may confer neuroprotection against the underlying dopaminergic neuron degeneration, and influence the onset and progression of PD. The available data also suggest that caffeine can improve the motor deficits of PD and that adenosine A_{2A} receptor antagonists such as istradefylline reduces OFF time and dyskinesia associated with standard 'dopamine replacement' treatments. Finally, recent experimental findings have indicated the potential of caffeine in the management of non-motor symptoms of PD, which do not improve with the current dopaminergic drugs. Altogether, the studies reviewed provide strong evidence that caffeine may represent a promising therapeutic tool in PD, thus being the first compound to restore both motor and non-motor early symptoms of PD together with its neuroprotective potential.

Keywords: Adenosine receptors, animal models, caffeine, learning and memory, motor deficits, neuroprotection, olfactory system, Parkinson's disease

INTRODUCTION

At the beginning of the nineteenth century, when James Parkinson first described the disorder that bears his name [1], life expectation was no longer than 45 years. Nowadays, life expectancy is near 80 years, and

the prevalence of Parkinson's disease (PD) is generally estimated at 0.3% of the entire population and about 1% in people over 60 years of age [2]. Since the incidence of the disease increases with age (the most important risk factor), it is likely that the number of people suffering from PD will rise steadily in the future.

Classically, PD is considered to be a motor system disease and its diagnosis is based on the presence of a set of cardinal motor signs (e.g., rigidity, bradykinesia, rest tremor and postural reflex disturbance). These symptoms of PD mainly result from the progressive de-

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generation of dopamine neurons of the substantia nigra pars compacta (SNc), which causes a consequent reduction of dopamine levels in the striatum [3]. Dopamine-replacement therapy has dominated the treatment of PD since the early 1960s and although the currently approved antiparkinsonian agents offer effective relief of the motor deficits, especially in the early stages of the disease, they have not been found to alleviate the underlying dopaminergic neuron degeneration, and drug efficacy is gradually lost [4]. Moreover, another major limitation of chronic dopaminergic therapy is the numerous adverse effects such as the development of abnormal involuntary movements (namely dyskinesia), psychosis and behavioral disturbance (e.g., compulsive gambling, hypersexuality) [5].

Dopamine replacement therapy is based on the importance of nigral dopaminergic cell loss and the ensuing striatal dopamine depletion for the onset of motor symptoms. However, the neurodegenerative processes that lead to sporadic PD begin many years before the appearance of the characteristic motor symptoms, and additional neuronal fields and neurotransmitter systems are also involved in PD, including the anterior olfactory structures, dorsal motor nucleus of vagus, caudal raphe nuclei, locus coeruleus, the autonomic nervous system, hippocampus, and the cerebral cortex [6] (Fig. 1). Accordingly, cholinergic, adrenergic, and serotoninergic neurons are also lost, which seems to be responsible for the non-motor symptoms of PD encompassing olfactory and memory impairments, sleep abnormalities, and depression, as well as gastrointestinal disturbance, which precede the classical motor symptoms [7]. Non-motor features of PD invariably do not respond to dopaminergic medication and are probably the major current challenge faced in the clinical management of PD [7].

Therefore, the limitations of the current pharmacological treatments of PD have led to extensive investigation of novel non-dopaminergic drugs that may provide alternative or adjunctive treatment for the relief of both motor and non-motor symptoms with a reduced profile of side-effects, as well as to the discovery of compounds to modify the course of PD. Over the last decade, several lines of evidence have suggested the potential of caffeine in the treatment of PD and an increasing number of studies have tested the potentially beneficial effects of caffeine (and more selective adenosine A_{2A} receptor antagonists) in different animal models and PD patients (Fig. 2).

Convergent epidemiological and pre-clinical data suggest that caffeine may confer neuroprotection
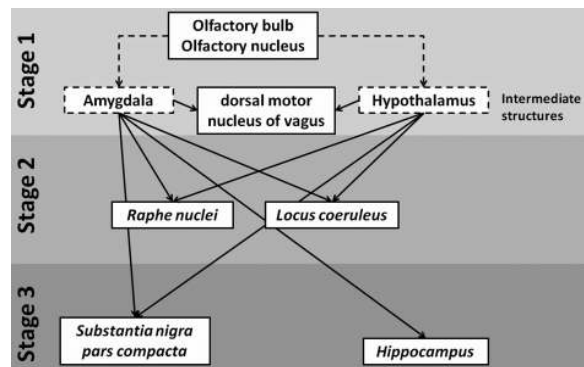

Fig. 1. Schematic illustration of the potential spread of dysfunction in different brain regions in Parkinson's disease during the first three stages, using the Braak's staging system [6], until the substantia nigra pars compacta is affected.

against the underlying dopaminergic neuron degeneration and can influence the onset and progression of PD. Indeed, the available data also suggest that caffeine, through the blockade of adenosine A_{2A} receptors in striatopallidal neurons, can improve the motor deficits of PD and that A_{2A} receptor antagonists such as istradefylline reduce OFF time and dyskinesia associated to standard 'dopamine replacement' treatments. Finally, recent experimental findings have indicated the potential of caffeine in the management of non-motor symptoms (e.g., depression, olfactory and memory dysfunction) of PD, which do not improve with the current dopaminergic drugs. The present review attempts to examine results reported in epidemiological, clinical and animal studies to provide a comprehensive picture of the antiparkinsonian potential of caffeine.

CAFFEINE AS A NEUROPROTECTIVE STRATEGY IN PARKINSON'S DISEASE

Current research on PD is largely devoted to investigating the etiology of the disease with the aim of identifying preventive rather than merely symptomatic treatments. Convergent epidemiologic and experimental evidence have suggested caffeine and selective adenosine A_{2A} receptor antagonists as novel potential strategies to attenuate dopaminergic neurodegeneration in PD. In a 30-year follow-up study of 8,004 Japanese-American men in the Honolulu Heart Program, Ross and colleagues [8] reported an inverse relationship between consumption of the non-selective adenosine antagonist caffeine and the risk of developing PD 20 years later. The age- and smoking-adjusted risk of PD was five times higher among men who reported no coffee
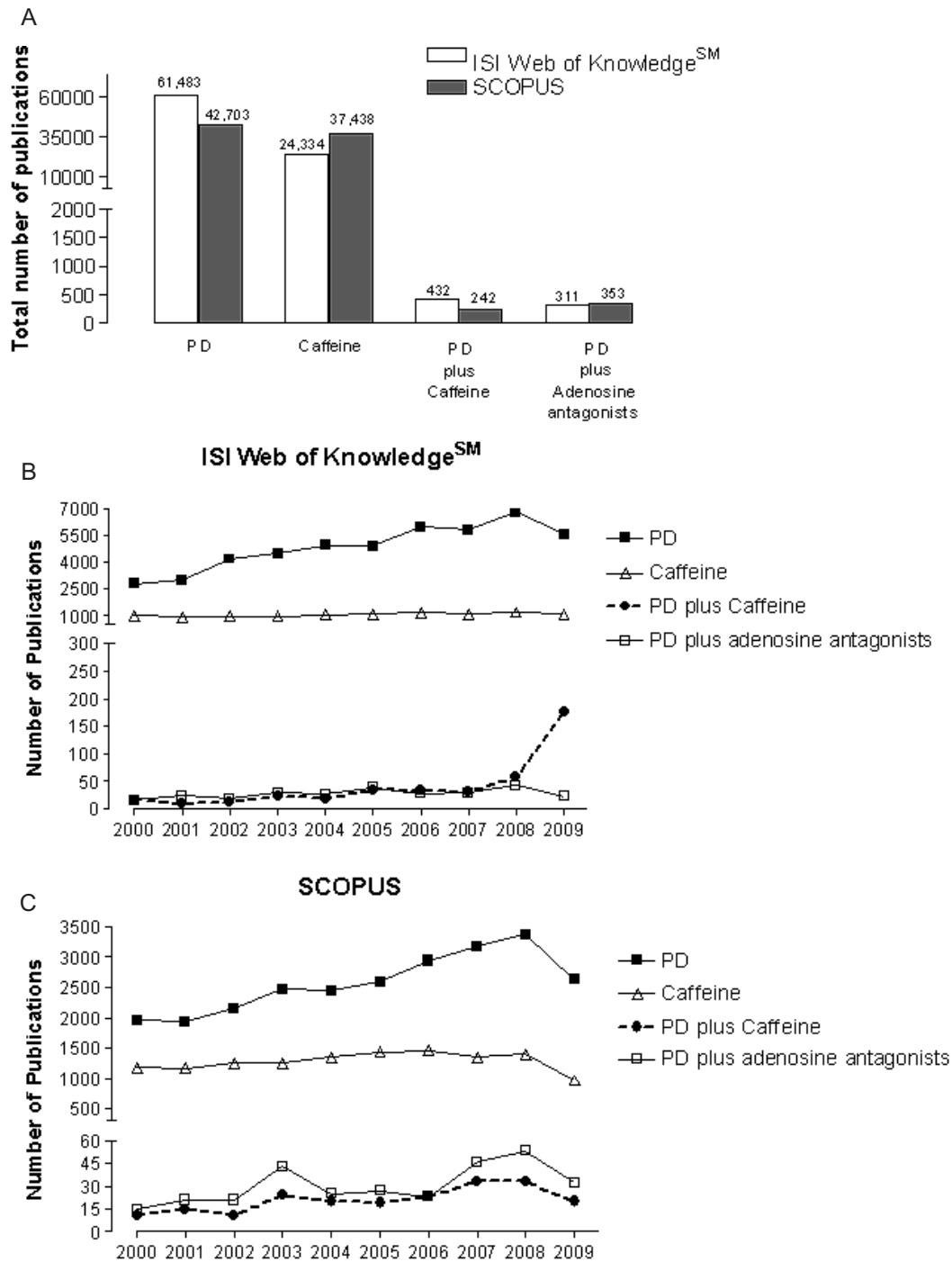
Fig. 2. Number of publications containing the keywords "Parkinson's disease" (PD), "caffeine", "PD plus caffeine" and "PD plus adenosine antagonists" found in the main databases analyzed. Panel A illustrates the total number of publications (time spam "all years") obtained for the queries comparing ISI Web of Knowledge^{SM} and SCOPUS. A time-line concerning the publications from year 2000 until October 23rd of the year 2009 obtained from ISI Web of Knowledge^{SM} and SCOPUS databases are shown in panels B and C, respectively. In the ISI Web of Knowledge^{SM}, data were generated using the keyword "Parkinson disease" rather than "Parkinson's disease", which caused a syntax error because of the apostrophe in this database; This did not occur with SCOPUS.

consumption compared with men who reported a daily consumption of 28 oz or more of coffee [8]. This finding was subsequently reinforced by further large prospective studies, which observed a similar inverse relationship between the consumption of caffeinated (but not decaffeinated) coffee and the risk of developing PD. These included the Health Professionals' Follow-Up Study and the Nurses' Health Study, involving 47,351 men and 88,565 women [9], and the Finnish Mobile Clinic Health Examination Survey, involving 19,518 men and women [10]. Men with consumption of tea and other caffeinated beverages had reduced risk of PD compared with men who were not regular caffeine drinkers ($<$ 1 cup/day), whereas no association was found with the consumption of decaffeinated coffee [8]. Interestingly, a 50% reduction of the risk of developing PD was observed among men with consumption of as low as one cup of caffeine per day when compared with men consuming no caffeine. These studies firmly established a relationship between increased caffeine consumption and the decreased risk of developing PD in men [8–10]. These findings raise the exciting possibility that caffeine as well as adenosine A_{2A} receptor antagonists may reduce the risk of developing PD by attenuating the degeneration of nigrostriatal dopaminergic neurons. In this context, caffeine has been included in a list of the twelve most promising neuroprotective agents for clinical trials in PD [11]. Curiously, in women, caffeine consumption is also linked to a reduced risk of PD but only among those who were not under hormone-replacement therapy [12]. Similar results have been found in an animal model of PD, where estrogen therapy prevented the neuroprotection by caffeine against nigrostriatal dopaminergic death [13].

The neuroprotective potential of caffeine in PD was further demonstrated in different animal models. Caffeine, when co-administered with 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) to mice at doses (5 to 30 mg/kg) comparable to the doses consumed by humans, dose-dependently attenuates the MPTP-induced loss of striatal dopamine neurons [14]. This protective effect of caffeine was observed with different MPTP exposure regiments (single and multiple doses) and in several mouse strains [14]. Furthermore, immunohistochemical analysis shows that caffeine can attenuate the MPTP-induced loss of nigral dopaminergic neurons [15]. Of high importance, caffeine-mediated neuroprotection apparently does not develop tolerance after continuous treatment, whereas nearly complete tolerance develops for its motor stimulant effect [16]. The

neuroprotective effects of caffeine have been attributed to an action on adenosine A_{2A} receptors since complementary studies with selective blockade of adenosine A_{2A} (but not A_1) receptors, through pharmacologic or genetic strategies, reported similar neuroprotective effects in the MPTP- [14,15,17] and 6-hydroxydopamine (6-OHDA) rodent models of PD [18]. However, no information is available to date as to whether the neuroprotective effects of caffeine or selective adenosine A_{2A} receptor antagonists can be extended to non-human primate models of PD. Nevertheless, the complimentary genetic and pharmacologic studies provide compelling evidence that caffeine reduces dopaminergic neurotoxicity in animal models of PD through the antagonism of adenosine A_{2A} receptors.

Although the exact molecular mechanisms underlying caffeine protection against the loss of dopaminergic neurons remains to be elucidated, it is important to emphasize that the neuroprotection by adenosine A_{2A} receptor antagonism extends beyond PD models [19, 20]. Chronic stressful stimuli cause an increased expression and density of adenosine A_{2A} receptors in animal models of PD [21,22], epilepsy [23], diabetes [24], and restraint stress [25]. Moreover, adenosine A_{2A} receptor antagonists confer neuroprotection against a broad spectrum of brain injury such as that induced by excitotoxicity [26–28], ischemia [29–33], and that associated with models of Huntington's disease [34] and Alzheimer's disease [35–37]. Therefore, the blockade of adenosine A_{2A} receptors confers neuroprotection in diverse brain regions ranging from substantia nigra, striatum to hippocampus and cortex, and against a variety of brain noxious stimuli.

Studies on neuroprotection exerted by caffeine and selective A_{2A} receptor antagonists are numerous and several mechanisms have been implicated in these effects. At this moment, particular attention is paid to the role of A_{2A} receptors in modulating glutamate release and glutamate uptake, as well as neuroinflammation (see Fig. 3), since these neurochemical changes have been associated with a variety of neuropathological processes, namely in PD models. Despite the multiple roles of glutamate in the brain as a protein constituent, neurotransmitter, and in the intermediary metabolism (for review see [38]), it can be highly toxic to neurons, a phenomenon known as "excitotoxicity", the process by which the over-activation of excitatory neurotransmitter receptors leads to neuronal cell death [39] through either rapid necrosis or delayed apoptosis of the neuron, depending on the severity of the insult [40]. In neurons, N-methyl-D-aspartic acid (NMDA) receptors,
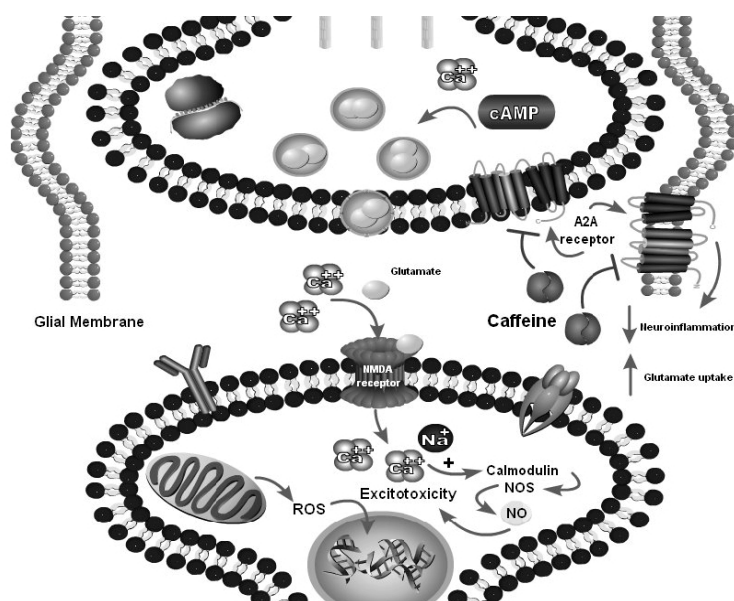
Fig. 3. Molecular mechanisms probably involved in the neuroprotective effects of caffeine and selective adenosine A_{2A} receptor antagonists. Adenosine A_{2A} receptors exert significant modulation of glutamate overflow by pre-synaptic and glial mechanisms and the blockade of adenosine A_{2A} receptors can represent a neuroprotective strategy by preventing glutamate excitotoxicity that is present in several neurodegenerative diseases including Parkinson's disease. Alternatively, the neuroprotection associated to the blockade of adenosine A_{2A} receptors may involve the modulation of neuroinflammation since activation of adenosine A_{2A} receptors produce complex effects in glial cells, including up-regulation of cyclo-oxygenase 2 and nitric oxide syntase, production of pro-inflammatory prostaglandins and cytokines and microglial activation.

which are highly permeable to calcium and distributed widely in the central nervous system (CNS) neurons, are the major initiators of excitotoxicity [41]. In PD, depletion of nigrostriatal dopamine results in disinhibition of striatal neurons which triggers glutamatergic overactivity [42].

As summarized in Fig. 3, adenosine A_{2A} receptors modulate glutamate release from nerve terminals and its clearance from astrocytes [19,43]. Several studies identified adenosine A_{2A} receptors as being responsible for the enhanced release of glutamate in noxious situations [28,44,45]. This hypothesis is particularly attractive because A_{2A} receptors are found in approximately 30% of glutamatergic pre-synaptic terminals in hippocampus [23], and because the activation of A_{2A} receptors can enhance glutamate release in the hippocampus and cortex of intact animals under normal and pathophysiological conditions [46]. More recently, adenosine A_{2A} receptor modulation of glial function has emerged as an additional mechanism by which A_{2A} receptor antagonists may modulate glutamate release and consequently neuronal cell death in CNS. Activation of adenosine A_{2A} receptors in cultured astrocytic glial cells derived from the cortex or brainstem was found to enhance glutamate efflux, whereas A_{2A} receptor blockade reduced the levels of extracellular glu-

tamate [47,48]. Thus, A_{2A} receptors exert significant modulation of glutamate overflow by pre-synaptic and glial mechanisms, and the blockade of adenosine A_{2A} receptors can be a neuroprotective strategy by preventing glutamate excitotoxicity that is present in several neurodegenerative diseases, including PD.

Another possible mechanism by which the treatment with caffeine and selective adenosine A_{2A} receptor antagonists confers neuroprotection may involve the modulation of neuroinflammation. The hallmark of neuroinflammation is glial (microglia and astrocytes) activation. Microglia, the resident immune cells in the brain, are sensitive to even minor disturbances in CNS homeostasis and become readily activated during most neuropathological conditions, such as PD, Alzheimer's disease, multiple sclerosis, AIDS dementia, trauma, and stroke (for recent review see [49]). Activated microglia cells are thought to contribute to neuronal damage via the release of pro-inflammatory and neurotoxic factors, such as tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β), reactive nitrogen species, proteases, reactive oxygen species, eicosanoids, and excitatory amino acids [49]. Intriguingly, in the mature brain the density of resting microglia in the substantia nigra is significantly higher than in other brain regions, which might be one of the reasons why dopamine-

containing neurons are extremely vulnerable to oxidative stress in PD [50]. An accumulation of extracellular adenosine in inflamed tissues has been reported [51,52]. However, such an anti-inflammatory action of adenosine in the brain is still a matter of debate [55]. Whereas the activation of adenosine A_{2A} receptors prevents peripheral inflammation [53,54], there is a paradoxical modulation of neuroinflammation by adenosine A_{2A} receptors [55]; in fact, when neuronal damage is the primary trigger of neuroinflammation, it is the blockade of A_{2A} receptors that prevents neuroinflammation [55].

This contradictory modulation by adenosine A_{2A} receptors can reflect the complexity of action of these receptors on neuronal, glial, and vascular components, which may have distinct effects in brain injury [20,55]. Apparently, activation of adenosine A_{2A} receptors produce complex effects in glial cells, including regulation of glutamate efflux (as described above), up-regulation of cyclo-oxygenase 2 and nitric oxide syntase, production of pro-inflammatory prostaglandins and cytokines, and microglial activation [56,57]. Consequently, activation of brain adenosine A_{2A} receptors might also induce potential deleterious effects by exacerbating neuroinflammatory process. Consistent with this notion, Pierry and colleagues [17] reported that the neuroprotection afforded by the selective adenosine A_{2A} receptor antagonist KW-6002 against MPTP neurotoxicity is associated with inhibition of microglial activation in the substantia nigra. More recently, Yu et al. [58] showed that MPTP administration increased the A_{2A} receptors density in microglia and astrocytes and that neuroprotection against dopaminergic neurotoxicity upon blockade of adenosine A_{2A} receptors was correlated with their ability to influence the MPTP-induced microglial and astrocytic activation.

A better understanding of how the multiple actions of A_{2A} receptors influence survival of dopaminergic neurons might further consolidate caffeine and selective adenosine A_{2A} receptor antagonists as potential neuroprotective agents for the treatment of PD.

CAFFEINE AS A PALLIATIVE TREATMENT FOR THE MOTOR IMPAIRMENTS IN PARKINSON'S DISEASE

A logical rationale to conceive a non-dopaminergic therapy for PD is to reverse the disruption to basal ganglia function by moving beyond the damaged dopaminergic input to the striatum and focusing on the activity of striatal output pathways, which is known to be im-

portant in the expression of motor symptoms and the onset and/or expression of dyskinesia [59]. At present time, there is an extensive literature demonstrating the ability of caffeine and selective adenosine A_{2A} receptor antagonists to modulate basal ganglia neurotransmission, which has been shown to be associated with improved motor function in diverse experimental models of PD [60,61]. In rodents, caffeine as well as selective A_{2A} receptor antagonists increase locomotor activity in MPTP-treated or reserpinized mice and reverse haloperidol-induced catalepsy [62,63]. A pioneering study by Fuxe and Ungerstedt [64] reported that caffeine induced contralateral turning in unilaterally 6-OHDA-lesioned rats. Subsequently other authors observed that the efficacy of caffeine in inducing turning behavior was observed only in 6-OHDA-lesioned rats receiving several priming doses of dopamine agonists, which in turn produced a supersensitive response to caffeine [65–67]. Indeed, the selective blockade of adenosine A_{2A} receptors by istradefylline or SCH 58261 increases contralateral rotation induced by L-DOPA or by stimulation of dopamine receptors in unilaterally 6-OHDA-lesioned rats [68–70]. Moreover, in 6-OHDA-lesioned rats rendered dyskinetic by prior treatment with L-DOPA, istradefylline produced an additive reduction in motor disability with L-DOPA, without worsening dyskinesia [71,72].

The classical triad of the cardinal motor features of PD includes muscle rigidity and tremor at rest in addition to bradykinesia (i.e., slow movements). Thus, additional features of A_{2A} receptor antagonists of particular clinical relevance would be an ability to relieve muscle rigidity and to counteract parkinsonian tremor. Correa et al. [73] have demonstrated that the adenosine A_{2A} receptor antagonist KF 17837 reverses the locomotor deficits and tremulous jaw movements induced by haloperidol in rats. Moreover, muscle rigidity (e.g., increased resistance to passive movement) induced by the dopamine-depleting agent reserpine or by the preferential dopamine D_2 receptor antagonist haloperidol can be reduced by the A_{2A} receptor antagonist SCH 58261 or eliminated by a synergistic combination of L-DOPA plus SCH 58261 [74].

The ability of adenosine A_{2A} receptor antagonists to improve motor disruption has been also documented in MPTP-treated non-human primates [75,76]. In MPTP-treated common marmosets, oral administration of istradefylline (0.5–100 mg/kg) increased locomotor activity for up to 9 h [75]. This effect was dose-dependent, with the greatest improvement observed at 10 mg/kg and with no additional benefit at higher dos-

es. A notable feature of A_{2A} receptor antagonists revealed by the experimental studies is their ability to enhance motor activity with reduced propensity to elicit dyskinesia [75,76]. For example, in L-DOPA-primed MPTP-treated cynomolgus monkeys, istradefylline at doses of 60 and 90 mg/kg significantly improved locomotor function, with an efficiency comparable to that observed with L-DOPA (50 mg/kg), but with little or no dyskinesia [76]. This observation raises the possibility that in PD patients with established dyskinesia, istradefylline monotherapy might provide motor benefit without eliciting dyskinesia. Additionally, chronic intermittent therapy using L-DOPA (or a dopamine agonist) can conspire with the hypodopaminergic state of PD to produce progressively briefer motor benefits and progressively more disruptive involuntary movements (dyskinesia) in response to each dose. The prevention and suppression of L-DOPA-induced dyskinesia have become major goals for the new non-dopamine approaches. Thus, the effect of adding istradefylline to L-DOPA or dopamine receptor agonists has been also investigated in non-human primate models of PD. For instance, the co-administration of istradefylline with threshold doses of L-DOPA and dopamine D_2 receptor agonists (such as quinpirole) provide an additive effect on motor function of MPTP-treated monkeys [77]. Moreover, Bibbiani and colleagues [78] have demonstrated that istradefylline completely prevents the development of dyskinesia induced by continuous apomorphine administration in MPTP-treated monkeys. Taken together, these pre-clinical studies performed in rodents and non-human primate models of PD have pointed to caffeine and selective adenosine A_{2A} receptor antagonists (e.g., istradefylline) as potential drugs to provide a relief of motor symptoms as monotherapy, as well as to potentiate the effects and to prevent the dyskinesia of chronic treatment with L-DOPA and dopamine receptor agonists.

These promising observations in animal models have led to clinical trials in PD patients with the caffeine-derived A_{2A} receptor antagonist (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002) [79, 80]. KW-6002 was shown to potentiate the symptomatic benefits conferred by a reduced dose of L-DOPA in relatively advanced PD and to produce motor enhancement that was comparable with that of an optimal L-DOPA dose [79,80]. Recently, istradefylline 40 mg/day was compared with placebo as monotherapy in early PD in a 12-week, double-blind, randomized, multi-center study involving 176 subjects [81]. The primary outcome measure was the change from baseline to endpoint in UPDRS motor scores. Although istradefylline provided numerical improvement over placebo, the difference in the primary outcome measure across groups was not statistically significant. These results differ from that obtained in pre-clinical studies in which caffeine and selective adenosine A_{2A} receptor antagonists reversed the motor deficits, suggesting that more exhaustive studies of istradefylline (or other adenosine A_{2A} receptor antagonists) monotherapy over longer periods and in a larger number of patients should be considered.

On the other hand, randomized, double-blind, placebo-controlled, phase II [82,83] and phase III [84, 85] clinical studies were subsequently conducted in PD patients on L-DOPA with motor fluctuations (with or without dyskinesia). The major finding of these recent clinical trials is that istradefylline (20–60 mg/day) reduces OFF time by 0.7–1.2 h. An increase occurs in ON time with dyskinesia, but most of this increase is non-troublesome dyskinesia [82,83,85]. However, Guttman [84] reported no significant reduction in OFF time versus placebo with istradefylline (10–40 mg/day) in PD patients with motor fluctuations, and the reason for this discrepancy is unknown.

Our understanding of how adenosine A_{2A} receptors modulate motor function is based on current models of basal ganglia anatomy and physiology. A detailed review of the anatomy, physiology, and biochemistry of the basal ganglia is beyond the scope of this article and can be found elsewhere [86]. The core components of the basal ganglia are the dorsal and ventral striatum and the globus pallidus (GP). The dorsal striatum is formed by the caudate nucleus and the putamen. Many authors refer to the ventral striatum as the nucleus accumbens (NAc), its main part. The GP consists of an internal (GPi) and an external (GPe) segment and of the ventral pallidum. Due to their reciprocal connections with these core structures, the substantia nigra, ventral tegmental area, and subthalamic nucleus (STN) are considered to be associated basal ganglia structures. The substantia nigra comprises two parts: the substantia nigra pars compacta (SNc), and the substantia nigra pars reticulata (SNr) [86,87]. They are thought to mediate the learning and processing of motor acts through the balance between two parallel polysynaptic pathways, the direct (striatonigral) and the indirect (striatopallidal) pathways (Fig. 4).

GABAergic neurons in the direct pathway project directly from the striatum to the GPi-SNr complex. They bear dopamine D_1 receptors and contain the peptides substance P and dynorphin. Striatal GABAergic neu-
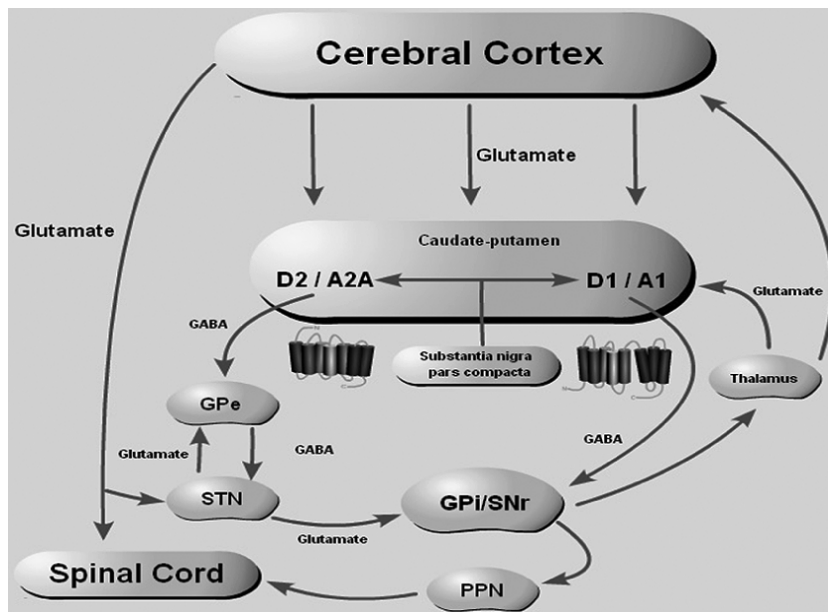
Fig. 4. Simplified diagram of the cortico-basal ganglia network and the possible mechanism by which caffeine and selective adenosine A_{2A} receptor antagonists improve parkinsonian motor dysfunction through their direct inhibitory influence on striatopallidal neurons, which co-express A_{2A} and D_2 receptors. The inhibitory influence of the striatonigral "direct" pathway on basal ganglia output (from the SNr/GPi complex) is counterbalanced by the disinhibitory influence of the striatopallidal "indirect" pathway to this complex. At the striatal level, dopamine, acting on D_1 receptors, facilitates transmission along the direct pathway and inhibits transmission along the indirect pathway throughout D_2 receptors. Adenosine enhances the activity of neurons in the indirect pathway via adenosine A_{2A} receptors in the striatum and globus pallidus pars externa (GPe). The progressive reduction of striatal dopamine levels in the course of Parkinson's disease (PD) results in an imbalance between the "indirect" and "direct" output pathways leading to increased inhibitory output from the internal GP (GPi) and substantia nigra pars reticulate (SNr) with excess inhibition of thalamocortical neurons, resulting in the reduced movement initiation characteristic of PD. Thus, adenosine A_{2A} receptor blockade in PD should result in recovery of GPe activity and decrease excitatory drive from the STN to the GPi-SNr complex, thereby restoring some balance between the direct and the indirect pathways. The schematic is adapted from Schwarzschild et al. [61]. See text for further citations.

rons in the indirect pathway influence the GPi-SNr indirectly via sequential synaptic connections in the GPe and then to the STN. This series of connections comprises: 1) an inhibitory GABAergic projection from the striatum to GPe; 2) an inhibitory GABAergic projection from the GPe to the STN (as well as to the GPi-SNr); and 3) an excitatory glutamatergic projection from the STN to the GPi-SNr. In contrast to striatonigral neurons, striatopallidal neurons express the D_2 subtype of dopamine receptors and contain the peptide enkephalin. However, the segregation of the direct and indirect pathways seems to be incomplete, with many projection neurons of the striatum expressing both D_1 and D_2 receptors [88]. In these cases, one family of dopamine receptors may predominate in each subpopulation of neurons.

At the striatal level, dopamine facilitates motor activity both by exciting D_1 receptor-expressing neurons in the direct pathway, and by inhibiting D_2 receptor-expressing neurons in the indirect pathway. In the striatum, adenosine A_{2A} receptors are mainly expressed on dopamine D_2 receptor-bearing striatopallidal neurons [60,61,87]. This unique cellular location of A_{2A} receptors provides an anatomical basis for the adenosine – dopamine interaction that underlies the motor symptomatic benefits of caffeine and selective adenosine A_{2A} receptor antagonists in PD (for review see [60, 61,87]). A large body of experimental evidence shows that adenosine A_{2A} receptor agonists inhibit dopamine D_2 receptor binding in striatum, D_2 receptor-mediated neurotransmitter release, and immediate early gene expression. Conversely, adenosine A_{2A} receptor antagonists mimic the effects of dopamine D_2 receptor agonists at cellular, neurochemical, and behavioral levels in normal and dopamine-depleted animals [87]. Therefore, the most likely mechanism by which caffeine and selective adenosine A_{2A} receptor antagonists improve parkinsonian motor dysfunction probably involves their direct inhibitory influence on striatopallidal neurons, which co-express A_{2A} and D_2 receptors.

In addition, experimental findings strongly suggest that, besides adenosine A_{2A} and dopamine D_2,

metabotropic glutamate 5 (mGlu5) receptors are also co-localized post-synaptically in the striatopallidal GABAergic efferent neuron [89]. This co-localization provides a structural framework for the existence of multiple functional interactions of A_{2A}, D_2 and mGlu5 receptors. A potentiation of motor activity has been reported upon combined administration of A_{2A} and mGlu5 receptor antagonists, together with a synergistic interaction at the level of signal transduction pathways [89–92]. The recent discovery of A_{2A}–mGlu5 heteromers in caudate-putamen (CPu) has further strengthened the rationale for studying antiparkinsonian strategies that simultaneously block adenosine A_{2A} and mGlu5 receptors [89]. Moreover, striatal cholinergic nerve terminals express adenosine A_{2A} receptors, and selective adenosine A_{2A} receptor antagonists can reduce the evoked release of acetylcoline in rat CPu [93]. This seems to represent a novel interesting target for tremor control in PD models.

Altogether, these observations emphasize the role of A_{2A} receptors in modulating dopaminergic, glutamatergic and cholinergic neurotransmission in basal ganglia and the potential of A_{2A} receptor antagonists for symptomatic treatment of motor impairments in PD.

CAFFEINE AS A PALLIATIVE TREATMENT FOR THE NON-MOTOR IMPAIRMENTS IN PARKINSON'S DISEASE

As stated in the Introduction, the neurodegenerative processes that lead to sporadic PD begin many years before the appearance of the characteristic motor symptoms and additional neuronal fields and neurotransmitter systems are also involved in PD [6] (Fig. 1). Accordingly, cholinergic, adrenergic and serotoninergic neurons are also lost and this seems to be associated with the non-motor symptoms of PD, which include olfactory and memory impairments, sleep abnormalities and depression, as well as gastrointestinal disturbances that precede the classical motor symptoms [7]. Non-motor features of PD invariably do not respond to dopaminergic medication and probably form the major current challenge faced in the clinical management of PD [7].

Subtle cognitive impairments consisting mainly of executive dysfunction with secondary visuospatial and mnemonic disturbances can be observed in the early stages of PD [94,95]. In about 20–40% of patients, these problems may eventually proceed to dementia, which constitutes an important risk factor for care-giver distress, decreased quality of life, and nursing home placement. Even non-demented PD patients have been reported to present visuospatial working memory deficits [94–97]. Furthermore, although there are reports of declarative (or episodic) memory impairments in PD [98], they are less severe in comparison to other neurodegenerative disorders such as Alzheimer's disease [95,98]. The fact that most of the drugs currently available for PD treatment (such as L-DOPA) are more efficient in alleviating motor rather than cognitive impairments has led many researchers to postulate non-dopaminergic mechanisms for the cognitive symptoms of PD [99,100]. On the other hand, animal models are an invaluable tool for studying the pathogenesis and progression of human diseases, as well as for testing new therapeutic intervention strategies. To date, most studies performed with animal models of PD have focused on their ability to induce nigrostriatal dopaminergic pathway damage and motor alterations associated with advanced phases of PD. Because PD is accompanied by alterations in a variety of functions, including anxiety disorders [101], memory deficits [94–100] and olfactory dysfunction [102–104], it seems important to evaluate whether the proposed animal models of PD display alterations of any of these functions. Until recently, no well-accepted model of the early phase of PD was available in the literature. This view has been re-evaluated following the findings that the infusion of MPTP by intranasal (i.n.) route [105–107] or directly into the rat SNc [108–110] causes a partial loss of dopamine neurons and depletion of striatal dopamine that result in olfactory and memory deficits. Corroborating clinical observations, the administration of benserazide/L-DOPA to MPTP-lesioned rats, at a dose that restores the striatal dopamine levels, fails to reverse MPTP-induced learning and memory impairment [110]. The failure of L-DOPA to improve memory deficits in both clinical studies and in bilaterally MPTP-lesioned rats reinforces the adequacy of this animal model to explore the potential of alternative drug therapies for the treatment of PD-related cognitive impairments.

As recently reviewed by Takahashi and colleagues [111], results obtained in different laboratories suggest that caffeine as well as selective adenosine A_1 and A_{2A} receptor antagonists can improve rodent learning and memory in diverse behavioral tasks [36,112–116]. However, few studies have specifically assessed the effects of caffeine or selective adenosine receptor antagonists on the cognitive impairment observed in animal models of PD. Gevaerd et al. [117] were

the pioneers to report that acute administration of caffeine reverses the impairing effect of MPTP-induced SNc lesion on the avoidance scores in the training and test sessions of a two-way active avoidance task in rats. Another animal model widely used for investigating symptomatic antiparkinsonian treatments is the systemic administration of reserpine, a drug that inhibits monoamine storage in intracellular granules, and hence causes their depletion in nerve terminals and induces transient hypolocomotion and muscular rigidity [118,119]. More recently, the use of low doses of reserpine (0.5–1.0 mg/kg) in rodents has been proposed as a behavioral approach to study the cognitive deficits [120–122] associated with PD. Prediger and collaborators [121] demonstrated that acute reserpine treatment (1.0 mg/kg, i.p.), 24 h before behavioral analysis, induces pronounced deficits in social recognition memory in rats. Interestingly, the reserpine-induced deficits in social recognition memory were reversed by acute administration (30 min prior to testing) of caffeine (10 or 30 mg/kg, i.p.) or the selective adenosine A_{2A} receptor antagonist ZM241385 (0.5 or 1.0 mg/kg, i.p.), but not by the adenosine A_1 receptor antagonist DPCPX (0.5 or 3.0 mg/kg, i.p.). However, these same authors had already shown that these doses of DPCPX prolong social recognition memory of non-reserpinized adult rats [115]. These results suggest an increased contribution of adenosine A_{2A} receptors (in detriment of A_1 receptors) in the cognitive deficits induced by reserpine administration in rats.

In addition, the presence of smell loss [102–104] and the pathological involvement of the olfactory pathways in the early stages of PD [6] are in accordance with the tenets of the olfactory vector hypothesis. This hypothesis postulates that some diseases such as PD may be caused or catalyzed by agents that enter the brain via the olfactory mucosa [123]. Consistent with this suggestion, we have recently proposed a new experimental model of PD consisting of a single i.n. administration of MPTP in rats [105,106] and mice [107]. Rodents treated intranasally with MPTP suffered progressive impairments in olfactory, cognitive, and motor functions associated with a time-dependent disruption of dopaminergic neurotransmission in different brain structures conceivably analogous to those observed during different stages of PD [105–107]. The MPTP-induced behavioral impairments in rodents are associated with alterations in the brain antioxidant status and lipid peroxidation [124] and apoptotic cell death mechanisms [106,107]. As can be seen in Fig. 5, rats infused intranasally with MPTP (1 mg/nostril) display impaired ability to recognize a juvenile intruder after a short period of time (30 min), spending as much time investigating the familiar juvenile rat during the second presentation as they did on the first encounter. This data corroborates early findings demonstrating that treatments that reduce the dopaminergic neurotransmission such as MPTP [107,125] and reserpine [121,122] inhibit short-term olfactory memory. Interestingly, the pre-treatment with caffeine (10 mg/kg/day, i.p.) during five consecutive days was able to prevent both the short-term social memory deficits and locomotor impairments observed at different periods after a single i.n. MPTP administration in rats (Fig. 5). Overall, from these limited results in this field, it appears that caffeine and selective adenosine A_{2A} receptor antagonists might be particularly useful to restore impaired learning and memory processes in MPTP and reserpine-treated rodents.

Beyond the memory symptoms, approximately 90% of PD patients at early stage exhibit olfactory dysfunction unrelated to the use of anti-PD medications (e.g., L-DOPA, dopamine agonists, anticholinergic compounds) [102]. Although no consistent evidence for the direct involvement of adenosine receptors in the olfactory deficits observed in PD has to date been documented, some indirect evidence allows one to speculate about the potential of caffeine and selective adenosine A_{2A} receptor antagonists to interfere with olfactory functions. Kaelin-Lang and colleagues [126] demonstrated an high expression of adenosine A_{2A} receptors in the granular cells of the accessory olfactory bulb of rodents. However, the functional importance of these receptors is still unknown. In addition, Hadfield [127] has demonstrated that caffeine, through the blockade of adenosine receptors, modulates the release of different neurotransmitters in the olfactory bulb of rodents, including dopamine, which plays a critical role in olfactory processing [128,129]. Furthermore, Prediger et al. [114] demonstrated that the acute administration of caffeine (10 and 30 mg/kg, i.p.) or the selective adenosine A_{2A} receptor antagonist ZM241385 (0.5 and 1.0 mg/kg, i.p.), but not the selective A_1 receptor antagonist DPCPX, improved the olfactory discrimination deficits in 12 month-old rats. Thus, this study provides the first evidence indicating that adenosine A_{2A} receptors play a critical role in the facilitatory effect of caffeine on olfactory discrimination in middle aged rats. Additional research is needed to clarify the mechanism underlying the blockade of adenosine A_{2A} receptors by caffeine which enhances the olfactory function in rodents.
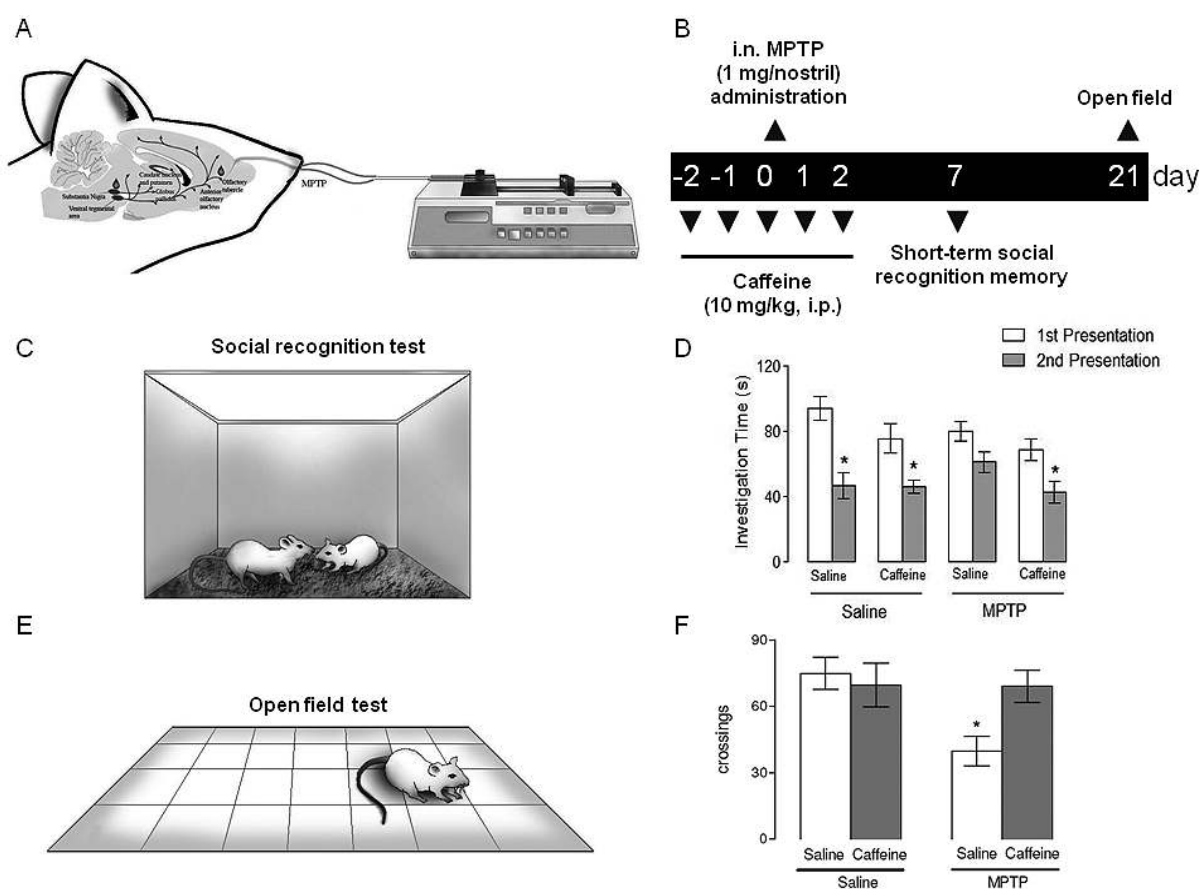
Fig. 5. (A) Schematic procedures of the intranasal (i.n.) administration of MPTP (1 mg/nostril) in rats [105,106]. (B) Time course of cognitive and motor behavioral tests evaluated in the intranasal MPTP rat model of Parkinson's disease. Pre-treatment with caffeine (10 mg/kg/day, i.p.) during 5 consecutive days prevented the (D) cognitive (social memory test) and (F) motor (open field test) impairments induced by i.n. MPTP administration in rats. *P<0.05 comparing saline and caffeine-treated groups.

Depressive disorders commonly occur in PD patients [101], affecting approximately 40% of patients at early stages of the disease [130]. Tadaiesky and coworkers [131] have demonstrated that that a partial striatal lesion with 6-OHDA induces depressive-like symptoms (e.g., anhedonia and behavioral despair) in rats. Cunha and colleagues [132] have recently reviewed the indirect evidence supporting a possible role of adenosine A_{2A} receptors in psychiatric disorders. Thus, because A_{2A} receptor antagonists might have anti-depressant properties [133], it will be important to consider whether motor and subjective improvement reflects elevation of mood in PD patients.

Although research is at a very early stage, the findings reviewed above highlight the memory-, olfactory- and mood-enhancing properties of caffeine in rodents. Therefore, the performance of additional pre-clinical and clinical studies to verify the effects of caffeine

and selective adenosine A_{2A} receptor antagonists in the non-motor symptoms of PD appears to be a promising field.

CONCLUSION

The findings presented in this review strongly suggest caffeine and selective adenosine A_{2A} receptor antagonists as non-dopaminergic candidates for symptomatic and potentially disease-modifying therapy in PD. Disease modification remains the most important goal in PD. Convergent epidemiological and pre-clinical data obtained in rodent models have suggested that caffeine may confer neuroprotection against the underlying dopaminergic neuron degeneration, and influence the onset and progression of PD. However, further studies are needed to demonstrate whether these

neuroprotective effects are also extensive to non-human primate models of PD. Moreover, the exact molecular mechanisms by which the blockade of adenosine A_{2A} receptors can protect dopaminergic neurons from degenerating remains to be elucidated. Indeed, the present data also suggest that caffeine, through the blockade of adenosine A_{2A} receptors in striatopallidal neurons, can improve the motor deficits of PD and that A_{2A} receptor antagonists such as istradefylline reduce OFF time and dyskinesia associated with standard 'dopamine replacement' treatments. Finally, recent experimental findings have indicated the potential of caffeine in the management of non-motor symptoms (e.g., olfactory, memory and psychiatric symptoms) of PD that do not improve with the current dopaminergic drugs. Altogether, the studies reviewed provide strong evidence that caffeine may represent a promising therapeutic tool in PD, thus being the first compound to restore both motor and non-motor early symptoms of PD, together with a neuroprotective potential.

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