



# Purinergic Signalling in Parkinson's Disease: A Multi-target System to Combat Neurodegeneration

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## Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by progressive loss of dopaminergic neurons that results in characteristic motor and non-motor symptoms. L-3,4 dihydroxyphenylalanine (L-DOPA) is the gold standard therapy for the treatment of PD. However, long-term use of L-DOPA leads to side effects such as dyskinesias and motor fluctuation. Since purines have neurotransmitter and co-transmitter properties, the function of the purinergic system has been thoroughly studied in the nervous system. Adenosine and adenosine 5'-triphosphate (ATP) are modulators of dopaminergic neurotransmission, neuroinflammatory processes, oxidative stress, excitotoxicity and cell death via purinergic receptor subtypes. Aberrant purinergic receptor signalling can be either the cause or the result of numerous pathological conditions, including neurodegenerative disorders. Many data confirm the involvement of purinergic signalling pathways in PD. Modulation of purinergic receptor subtypes, the activity of ectonucleotidases and ATP transporters could be beneficial in the treatment of PD. We give a brief summary of the background of purinergic signalling focusing on its roles in PD. Possible targets for pharmacological treatment are highlighted.

**Keywords** Adenosine · Adenosine receptors · ATP · Parkinson's disease · Purinergic receptors

## Abbreviations

ADORA2A	Adenosine A <sub>2A</sub> receptor	GABA	γ-Amino butyric acid
ADP	Adenosine 5'-diphosphate	GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A
AP4A	Diadenosine tetraphosphate	5-HT <sub>1A</sub>	5-Hydroxytryptamine/serotonin receptor 1A
ATP	Adenosine 5'-triphosphate	L-DOPA	L-3,4 dihydroxyphenylalanine
cAMP	Cyclic adenosine monophosphate	LPS	Lipopolysaccharide
CB <sub>1</sub>	Cannabinoid receptor type 1	LRRK2	Leucine-rich repeat kinase 2
DA	Dopamine	6-OHDA	6-hydroxydopamine
		mGlu	Metabotropic glutamate receptor
		MPP <sup>+</sup>	1-Methyl-4-phenylpyridinium
		MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
		MSA	Multiple system atrophy
		NMDA	N-methyl-D-aspartate
		PD	Parkinson's disease
		ROS	Reactive oxygen species
		UDP	Uridine 5'-diphosphate
		UTP	Uridine 5'-triphosphate

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## Introduction

### Parkinson's Disease: Pathophysiological Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* that results in dopamine (DA) deficiency in the striatum. The ongoing degeneration of this peculiar pathway causes the characteristic motor symptoms such as resting tremor, rigidity, bradykinesia and postural instability [1, 2]. Besides dopaminergic neural degeneration, the presence of Lewy bodies (protein aggregates) due to misfolding of  $\alpha$ -synuclein occurs in various regions of the affected brain [3]. In spite of many studies on the pathogenesis of PD, the precise mechanism underlying these events has not been unraveled yet. However, a genetic predisposition associated with disturbed proteostasis due to impaired ubiquitin–proteasome system, mitochondrial dysfunction, oxidative stress and neuroinflammation seems to play cardinal roles for the  $\alpha$ -synuclein aggregation and the progression of pathology in PD [4–7]. Among these factors, the pathological, self-amplifying interaction between mitochondrial dysfunction and oxidative stress has been early recognized, which might be a key factor responsible for the selective vulnerability of dopaminergic neurons in PD, and one potential reason behind the clinical failures of neuroprotective therapies so far [8]. Dysfunction of the mitochondrial complex I results in an enhanced production of reactive oxygen species (ROS), which, in turn will inhibit complex I and other vital metabolic enzymes such as alpha-ketoglutarate dehydrogenase, whilst the latter also serves as a source of ROS generation in mitochondria [9, 10]. Simultaneous or preceding mitochondrial dysfunction exacerbates the effect of oxidative stress on pathological monoamine release from nerve terminals [11, 12]. This process leads to the formation of toxic, oxidative DA metabolites, such as dopamine quinone, which might further amplify the ongoing degeneration process [13]. Therefore, disease-modifying potential could be primarily expected from those novel multi-target therapies, which simultaneously target the above mentioned pivotal pathological pathways and prevent their pathological interaction [14, 15].

### The Current Treatment of PD

As for the symptomatic treatment of PD, the clinical breakthrough came with the first clinical trials of DA replacement therapy using the high dosage of the DA precursor

L-3,4 dihydroxyphenylalanine (L-DOPA) [16–19]. L-DOPA is able to cross the blood–brain barrier and converts into DA that engages specific DA receptor subtypes ( $D_1$  to  $D_5$ ) [20]. However, long-term use of L-DOPA leads to a dysbalance of striatal circuits of the motor system and leads to side effects such as L-DOPA induced dyskinesias and motor fluctuation in 50% of patients after 5 years of continuous treatment [21, 22]. The therapeutic management of these complications is difficult and there is a need for developing effective and new pharmacological therapies against motor fluctuation and dyskinesias [23].

### Purinergic Signalling: Concept and Purinergic Receptors

The concept of purinergic signalling, being adenosine 5'-triphosphate (ATP) as an extracellular signalling molecule with neurotransmitter properties was proposed in the early 1970s [24, 25]. A couple of years later, purines were also described as co-transmitters and neuromodulators in the peripheral and central nervous system (CNS), as they are able to modulate other signalling pathways and neurotransmitter systems [26–28]. ATP is co-released with acetylcholine, catecholamines,  $\gamma$ -amino butyric acid (GABA), glutamate and DA in the CNS [29–34]. Extracellular ATP is released from cells under physiological conditions. The levels of extracellular ATP are controlled by ectonucleotidases that catalyze its degradation [35, 36].

There are two families of purinergic receptors, which are distinguished by their main agonists [37]. P1 receptors are G protein-coupled metabotropic receptors activated by adenosine and can be subdivided into four subtypes ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ ,  $A_3$ ). P2 receptors are subdivided into two classes: P2X<sub>(1-7)</sub> ionotropic receptors, activated by ATP and G protein-coupled metabotropic P2Y<sub>(1-2,4,6,11-14)</sub> receptors, activated by ATP, adenosine diphosphate (ADP), uridine di- and triphosphate (UDP and UTP), or UDP-glucose depending on the receptor subtype [38–40]. ATP is able to bind to the extracellular ligand-binding site of P2X receptors and leading to conformational change that opens a permeable channel to  $Na^+$ ,  $K^+$  and  $Ca^{2+}$ . The activation of these ionotropic receptors is important for  $Ca^{2+}$ -induced intracellular signalling pathways [41–43]. Depending on the activated adenosine and P2 receptor subtype, the induced signalling pathway may vary. These activated receptors are able to make alterations in  $Ca^{2+}$  levels, which modulate the activity of several secondary messengers involved in physiological processes [44–46]. The final effects of purinergic receptor-mediated signalling depend on the cell type and other physiological (neurogenesis, proliferation, cell death, stem cell differentiation) or pathological cellular conditions (inflammatory, neurological, psychiatric, oncological, cognitive, neuromuscular and neuromotor diseases) [47–66]. Purinergic receptor

activation may have para- or autocrine nature, which is characteristic for astrocytes in the regulation of neuronal activity [67]. Not only purinergic receptors but membrane nucleotide/nucleoside transporters, channels and ectonucleotidases also play important role in purinergic signalling [36, 68–70].

Adenosine is the predominant, presynaptic modulator of neurotransmitter release in the CNS, although ATP has presynaptic modulator effect as well [71–73]. Adenosine is produced by enzymatic breakdown of released ATP, but some CNS cells are able to release adenosine directly [74]. A<sub>1</sub> and A<sub>2A</sub> receptors have higher affinity (activated by physiological extracellular levels of adenosine) and A<sub>2B</sub> and A<sub>3</sub> receptors have lower affinity (activated by higher extracellular levels of adenosine) for the ribonucleoside [75–77]. The adenosine A<sub>1</sub> and A<sub>2A</sub> receptors are highly expressed in the brain and CNS, where they have profound influence on neuronal activity. Adenosine A<sub>1</sub> receptor is the dominant adenosine receptor subtype in the CNS. Adenosine A<sub>1</sub> receptors can be found in various cortical and subcortical regions of the brain, while A<sub>2A</sub> receptors are mainly expressed in the striatum [78–81] (Table 1). In contrast, adenosine A<sub>2B</sub> and A<sub>3</sub> receptors are mainly found in peripheral tissues, even though low levels of these receptors are also expressed in some regions of the brain [82–84].

There is a heterogeneous distribution of P2 purinergic receptors in the CNS as well. For instance P2X<sub>1</sub> receptors are predominantly expressed in the cerebellum, while P2X<sub>3</sub> receptors are expressed in the brainstem [85, 86], and they can be found in the basal ganglia with variable expression level [87] (Table 2). Various P1 and P2 receptor subtypes are also expressed by microglia, astrocytes and oligodendrocytes [88–93]. Extracellular nucleotides act as messengers between neuronal and non-neuronal cells, thereby integrating functional activity between neurons, glial and vascular cells in the CNS [94–98]. Adenosine and ATP—as key players in neuron–glia interaction and microglial activation—are modulators of neuroinflammatory processes, oxidative stress, excitotoxicity and cell death [99–102]. Aberrant purinergic receptor signalling can be the cause or result of numerous pathological conditions, including neurodegenerative disorders [103]. Here, we explore the importance of purinergic signalling in PD to suggest potential targets for novel therapies.

**Table 2** Expression of P2 receptor subtypes in the basal ganglia (striatum and substantia nigra) [87]

	Striatum	Substantia nigra
P2X <sub>1</sub>	↑↑↑	↑↑↑
P2X <sub>2</sub>	↑↑↑	↑↑↑
P2X <sub>3</sub>	↑↑	↑↑
P2X <sub>4</sub>	↑↑↑	↑↑↑
P2X <sub>5</sub>	↑	↑↑↑
P2X <sub>6</sub>	↑	↑↑
P2X <sub>7</sub>	↑↑	↑↑
P2Y <sub>1</sub>	↑	↑↑
P2Y <sub>2</sub>	↑↑↑	↑↑↑
P2Y <sub>4</sub>	↑↑↑	↑↑↑
P2Y <sub>6</sub>	↑	↑↑↑
P2Y <sub>11</sub>	–	–
P2Y <sub>12</sub>	↑↑↑	↑↑↑
P2Y <sub>13</sub>	–	–
P2Y <sub>14</sub>	–	↑↑↑

Expression level of P2 receptor subtypes: – = no expression, ↑ = low expression, ↑↑ = medium expression, ↑↑↑ = high expression

**Table 1** Localization of adenosine receptor subtypes in CNS [80, 81]

	CNS
A <sub>1</sub>	High levels in striatum, thalamus and moderate levels in cortex, pons
A <sub>2A</sub>	High levels in striatum, thalamus, hippocampus
A <sub>2B</sub>	Low levels in microglia cells, astrocytes
A <sub>3</sub>	Low levels in cortex, hippocampus, striatum, cerebellum

## Purinergic Signalling Involvement in PD

### Purinergic Gene Polymorphisms in PD

Two *ADORA2A* (A<sub>2A</sub> receptor) polymorphisms (rs71651683, a 5' variant or rs5996696, a promoter region variant) were inversely associated with genetic PD risk, moreover, there was evidence of interaction with coffee consumption [104]. *CYP1A2a* is an enzyme, which is responsible for caffeine metabolism, two polymorphisms (rs762551 or rs5996696) of the enzyme in homozygous coffee drinkers reduced PD risk [104]. Humans with R1628P variant (LRRK2 risk variant) who did not take caffeine had a 15 times increased risk of PD [105]. *GRIN2A* encodes an N-methyl-D-aspartate-2A (NMDA) glutamate receptor subunit involved in central excitatory neurotransmission, which is associated with A<sub>2A</sub> receptor activation. Carriers of *GRIN2A* rs4998386-T allele had a lower risk of PD, than carriers of rs4998386-CC

genotype among heavy coffee drinkers [106]. There is evidence that creatine is able to hasten PD progression in *GRIN2A* coffee drinkers, which demonstrates an example of a genetic factor interacting with environmental factors exemplifying the complexity of environment–gene interactions in the progression of PD [107]. In addition, P2X<sub>7</sub> receptor 1513A>C polymorphism is a risk factor for sporadic PD, late-onset PD and male PD in Han Chinese population [108].

### Adenosine Receptor-Mediated Signalling in PD

A<sub>2A</sub> receptors are enriched in dopaminergic brain areas (the highest expression of these receptors are in the striatum), thus pointing to a significant role of purines in motor control [109]. A<sub>2A</sub> and DA D<sub>2</sub> receptors are mainly expressed in the neurons of the indirect pathway of striatal circuits projecting to the *globus pallidus*, in contrast to A<sub>1</sub> and DA D<sub>1</sub> receptors, which are mainly found on the neurons of the direct pathway of motor control projecting to the internal *globus pallidus* and *substantia nigra pars reticulata*. The main adenosine signalling mechanism is via the cyclic adenosine monophosphate (cAMP)-dependent pathway. Activated A<sub>2A</sub> receptors stimulate the enzymatic function of adenylyl cyclase that increases cAMP levels and depresses the signalling mediated by D<sub>2</sub> receptors. Activation of protein Gi-coupled DA D<sub>2</sub> receptors leads to reduction in the cAMP level. There is a reciprocal situation in the direct pathway of motor control with protein Gs-coupled D<sub>1</sub> and protein Gi/o-coupled A<sub>1</sub> receptors. Generally, adenosine acts as a negative modulator of D<sub>1</sub>- and D<sub>2</sub>-mediated actions in the direct and indirect pathways [110–112].

The antagonistic functional interaction between adenosine A<sub>2A</sub> and DA D<sub>2</sub> receptors may depend on the formation of receptor heterodimers (A<sub>2A</sub>-D<sub>2</sub> heteroreceptor complexes) in the striatum thereby balancing the inhibitory and excitatory impulses in the striatal circuits [112]. Not only dopaminergic mechanisms, but non-dopaminergic modes of action of A<sub>2A</sub> receptors may involve interactions with various non-dopaminergic receptors, possibly by forming heterodimeric and/or multimeric receptor complexes [23]. Thus, adenosine A<sub>2A</sub> receptors may adjust the actions of striatal adenosine A<sub>1</sub> receptors (A<sub>1</sub>-A<sub>2A</sub> heteroreceptor complexes), metabotropic glutamate receptors (mGlu) 5 (A<sub>2A</sub>-mGlu<sub>5</sub> heteroreceptor complexes), cannabinoid receptor type 1 (CB<sub>1</sub>) receptors (A<sub>2A</sub>-CB<sub>1</sub> heteroreceptor complexes) and serotonin 1A (5-HT<sub>1A</sub>) receptors [113–115]. Moreover, studies also suggested the presence of multimeric A<sub>2A</sub>-D<sub>2</sub>-mGlu<sub>5</sub> and A<sub>2A</sub>-CB<sub>1</sub>-D<sub>2</sub> receptor complexes in the striatum [116, 117]. These functional interactions between receptors may modulate the activity of striatal efferent neurons and influence motor behavior [23]. In general, adenosine tone appears as a key for the fine tune control of DA dependent actions in

the basal ganglia and affects non-dopaminergic mechanisms also [20].

Adenosine receptor antagonists (especially non-selective A<sub>2A</sub> receptor antagonists, such as methylxanthines, caffeine, or selective A<sub>2A</sub> antagonists) have been shown to enhance therapeutic effect of L-DOPA in a wide range of animal models of PD [118–121]. A<sub>2A</sub> homoreceptor complexes are in balance with DA D<sub>2</sub> homoreceptor complexes in intact striatum [122–126]. Dysbalance of striatal circuits leads to motor inhibition and disruption of this balance in PD leads to increased signalling via A<sub>2A</sub> receptors and decreased signalling via DA D<sub>2</sub> receptors. These changes explain the beneficial effect of A<sub>2A</sub> receptor antagonists on increasing motor functions without worsening L-DOPA-induced dyskinesias [20, 127].

A<sub>2A</sub> receptor antagonists have been used in clinical trials in patients with PD (Table 3). Istradefylline is a xanthine-based compound with increased selectivity for A<sub>2A</sub> receptors against A<sub>1</sub> receptors, which is used concomitantly with L-DOPA [128]. The drug was not approved in the USA because there was no significant reduction in *off time* compared to L-DOPA treatment [129]. In contrast, istradefylline was approved in Japan in 2013 with the trade name Nourias<sup>®</sup> to enhance the antiparkinsonian effect of L-DOPA with less long-term side effects [130, 131]. Preladenant is a second-generation A<sub>2A</sub> receptor antagonist, which failed in phase III clinical trials in the treatment of PD because the compound was not superior to placebo in reducing *off state* [132, 133]. Vipadenant is a triazolopyrimidine-based drug, which has increased selectivity for A<sub>2A</sub> receptors versus A<sub>1</sub> and A<sub>3</sub> receptors [134]. Its development as an antiparkinsonian medication was stopped; however, A<sub>2A</sub> receptor antagonists have considerable potential in novel immune-oncology and cardiology therapies [113, 135–137]. Another adenosine A<sub>2A</sub> receptor antagonist, tozadenant was safe, well tolerated and effective in reducing *off time* in PD patients in phase II trial but phase III clinical trial was discontinued because of serious adverse events (agranulocytosis) [23, 133, 138]. There have been many drug trials for selective A<sub>2A</sub> receptor antagonists. Most of them were shown to be safe, well tolerated and beneficial; however, the majority did not reach the regulatory threshold for efficacy to be approved as PD drugs [139, 140]. Development of bivalent drugs (able to bind to two receptors simultaneously) to target A<sub>2A</sub>-D<sub>2</sub> heteroreceptor complexes acting on A<sub>2A</sub> and DA D<sub>2</sub> receptors may be a good therapeutic approach in the future. Heterobivalent drugs offers the opportunity to target the orthosteric sites of the receptors in the heterodimer with a higher affinity and a higher specificity versus corresponding homomers and reduce the dose required for therapy and, accordingly, the side effects [20].

Adenosine A<sub>2A</sub> receptor antagonists may also involve direct or indirect actions at microglia and inflammatory

**Table 3** Pre-clinical and clinical studies with purinergic receptor antagonists in PD

Compounds	Mechanism of effect	Models	Published	Results
KW-6002 (istradefylline)	A <sub>2A</sub> receptor antagonism	PD patients	2003	Improved PD motor scores when added to low-dose L-DOPA
KW-6002 (istradefylline)	A <sub>2A</sub> receptor antagonism	LPS treated rats	2013	Enhanced therapeutic effect of L-DOPA
Caffeine	A <sub>2A</sub> receptor antagonism	LPS treated rats	2013	Reduced motor impairment
Preladenant	A <sub>2A</sub> receptor antagonism	MPTP treated mice	2014	Enhanced therapeutic effect of low doses of L-DOPA
8-Ethoxy-9-ethyladenine	A <sub>2A</sub> receptor antagonism	6-OHDA lesioned rats	2015	Enhanced effect of low doses of L-DOPA without increased dyskinesia
SCH 58261	A <sub>2A</sub> receptor antagonism	A <sub>2A</sub> receptor knockout mice, SH-SY5Y cells	2015	Decreased $\alpha$ -synuclein aggregation, prevented neuronal death
ZM 241385	A <sub>2A</sub> receptor antagonism	A <sub>2A</sub> receptor knockout mice, SH-SY5Y cells	2015	Decreased $\alpha$ -synuclein aggregation, prevented neuronal death
Preladenant	A <sub>2A</sub> receptor antagonism	PD patients	2017	Failed (was not superior to placebo) in phase III clinical trial
Vipadenant	A <sub>2A</sub> receptor antagonism	PD patients	2009	Failed (was not superior to placebo)
Tozadenant	A <sub>2A</sub> receptor antagonism	PD patients	2017	Failed in phase III clinical trial (induced agranulocytosis)
NF449	P2X <sub>1</sub> receptor antagonism	H4 cells	2015	Prevented $\alpha$ -synuclein aggregation
A-438079	P2X <sub>7</sub> receptor antagonism	6-OHDA lesioned rats	2010	Prevented depletion of DA in striatum
BBG	P2X <sub>7</sub> receptor antagonism	6-OHDA lesioned rats	2014	Reverted dopaminergic neurons loss in substantia nigra
BBG	P2X <sub>7</sub> receptor antagonism	BV2 microglia cells	2015	Decreased ROS production induced by $\alpha$ -synuclein
PPADS	P2X <sub>7</sub> receptor antagonism	SH-SY5Y cells	2017	Prevented abnormal calcium influx induced by $\alpha$ -synuclein
AZ 11645373	P2X <sub>7</sub> receptor antagonism	SH-SY5Y cells	2017	Prevented abnormal calcium influx induced by $\alpha$ -synuclein
AP4A	P2Y <sub>2</sub> /P2Y <sub>4</sub> antagonism	6-OHDA lesioned rats	2003	Reduced dopaminergic neurons loss
MRS2578	P2Y <sub>6</sub> receptor antagonism	SH-SY5Y cells	2017	Delayed neuronal loss

The list is not comprehensive and is restricted to studies mentioned in the article. For further references, see [111, 113]

processes. Pre-treatment of slices from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-injected mice with preladenant facilitates the ability of activated microglia to respond to tissue damage [141]. The nonselective A<sub>1</sub>/A<sub>2A</sub> adenosine receptor antagonist caffeine and the selective A<sub>2A</sub> receptor antagonist (KW-6002) had anti-inflammatory potential in a rat model of lipopolysaccharide (LPS)-induced neuroinflammation [142].

### The Role of A<sub>2A</sub> Receptors in Synucleopathy

Increased striatal A<sub>2A</sub> receptor expression was observed as an early pathological event in PD and increased A<sub>2A</sub> receptor expression was detected after hippocampal injection of  $\alpha$ -synuclein in mice [143, 144]. A<sub>2A</sub> receptor-knock out mice showed resistance against  $\alpha$ -synuclein induced insults [145]. A<sub>2A</sub> receptor antagonism restrained hyperactivation of NMDA-glutamate receptors and decreased the aggregation of  $\alpha$ -synucleins [146]. Based upon these results, A<sub>2A</sub>

receptors seem to have role in the pathological process of synucleinopathy [111].

### P2 Receptor-Mediated Signalling in PD

P2 ionotropic and metabotropic receptors are widely expressed in basal ganglia and in various cell types, such as neurons and astrocytes [87, 147, 148]. 6-Hydroxydopamine (6-OHDA) induced lesions of nigral dopaminergic neurons generate a significant decrease in the expression of P2X and P2Y receptor proteins from striatal spiny neurons and GABAergic interneurons, thus confirming the involvement of P2 receptors and extracellular ATP in the striatal circuits [87]. P2Y<sub>1</sub> and P2X<sub>1-4,6</sub> receptor protein subtypes are expressed in dopaminergic neurons with co-expression of P2X<sub>1</sub> with DA D<sub>1</sub> receptors, therefore stimulation of P2 receptors by ATP induces an increased release of DA in the striatum [149–152]. In a neuronal cell model, extracellular ATP induced a significant increase in intracellular

$\alpha$ -synuclein levels, which was the result of lysosome dysfunction caused by P2X<sub>1</sub> receptor activation [153].

Many data have implicated the role of P2X<sub>7</sub> receptor in PD. P2X<sub>7</sub> receptor antagonism with A-438059 or Brilliant Blue G (BBG) prevented DA deficit in the striatum and 6-OHDA-induced hemiparkinsonian behavior [154, 155]. However, P2X<sub>7</sub> receptor deficiency or inhibition did not promote the survival of dopaminergic neurons in rotenone and MPTP induced animal models of PD [156]. It is presumed that there is a massive release of ATP during cell death in the lesioned striatum and *substantia nigra*, which activates cell death pathways via purinergic receptors and is able to activate further purinergic subtypes [20]. Permanent purinergic receptor activation and ATP release seem to play a key role in the neuronal death, which exacerbates  $\alpha$ -synuclein aggregation in PD [87]. The accumulation of  $\alpha$ -synuclein might overwhelm the capacity of intracellular protein-degradation mechanisms and induce neuroinflammation, which creates a positive feedback loop promoting the degeneration of dopaminergic cells [7].  $\alpha$ -Synuclein-induced intracellular free calcium mobilization in neuronal cells depends on the activation of purinergic P2X<sub>7</sub> receptors. In the same study, activation of P2X<sub>7</sub> receptors lead to ATP release with the recruitment of the pore forming protein pannexin1, whilst  $\alpha$ -synuclein decreased the activity of extracellular ecto-ATPase which is responsible for ATP degradation [157]. Stimulation of the microglial P2X<sub>7</sub> receptor by extracellular  $\alpha$ -synuclein increased oxidative stress, which was prevented with the use of P2X<sub>7</sub> receptor antagonist [158].

DA neurotransmission has been linked to calcium signalling. There is data that P2Y<sub>1</sub> receptor is involved in the regulation of calcium signalling [159]. Neurodegeneration induced by 6-OHDA in nigrostriatal dopaminergic neurons was reduced by pretreatment with diadenosine tetraphosphate (AP4A, an endogenous diadenosine polyphosphate) possibly through an anti-apoptotic mechanism and the activation of P2Y<sub>1</sub> and P2Y<sub>4</sub> receptors [160]. Recently, expression levels of P2Y<sub>6</sub> receptor in PD patients younger than 80 years were higher than healthy controls and multiple system atrophy (MSA) patients and P2Y<sub>6</sub> receptor could thereby be a potential clinical biomarker of PD. P2Y<sub>6</sub> receptor was also upregulated in LPS-treated microglial cells and involved in proinflammatory cytokine release through UDP secretion [161]. Another study showed that expression of P2Y<sub>6</sub> receptor on neuronal SH-SY5Y cell is associated with the progression of oxidative stress and cell death induced by 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) [162]. In vivo, LPS induced microglial activation and delayed neuronal loss was prevented by selective inhibition of P2Y<sub>6</sub> receptor with MRS2578 [163]. Based on these studies P2Y<sub>6</sub> receptor subtype seems to be involved in the process of neuroinflammation in PD and blocking UDP/P2Y<sub>6</sub> receptor signalling could reverse these pathological processes [161].

## Conclusion

In general, many data confirm the involvement of purinergic signalling pathways in PD. Modulation of purinergic receptor subtypes, the activity of ectonucleotidases and ATP transporters could be beneficial in the treatment of PD. Antagonism of A<sub>2A</sub>, P2X<sub>1</sub>, P2X<sub>7</sub> and P2Y<sub>6</sub> receptor subtypes is a promising weapon against PD via various ways: reducing L-DOPA induced dyskinesia, influencing neuroinflammation, preventing  $\alpha$ -synuclein aggregation, reducing microglia activation. Development of new bivalent compounds to target A<sub>2A</sub>-D<sub>2</sub> heteroreceptor complexes, which are orally bioavailable and can cross the blood–brain barrier could be a potential therapeutic tool. In addition, multi-target compounds targeting self-amplifying circuits controlled by purinergic and non-purinergic receptors could be a viable strategy to obtain the desired disease-modifying effect [164]. Additional studies and better quality PD animal models are required for the deeper understanding of underlying unknown pathological processes in PD and the role of purinergic signalling in it.

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