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How can caffeine alleviate the motor symptoms of Parkinson's disease? – the implications of adenosine 2A receptor antagonism

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Abstract

Introduction and purpose: Parkinson's disease (PD) is the second most common neurodegenerative disease, mainly characterized by motor impairment with symptoms including rigidity, bradykinesia, rest tremor, and imbalance. It develops upon degeneration of dopaminergic neurons in the substantia nigra which is associated with the neuroinflammatory process initiated by alpha-synuclein deposits. Although, levodopa replacement therapy is the gold-standard treatment, the majority of the treated patients develop dyskinesia as a side effect, resulting from altered function of dopamine receptors. It is thought that the abnormal pulsate release of dopamine can be prevented by antagonism of adenosine 2A receptors (A2ARs).

Aim of the study: This review aims to outline the implications of A2AR antagonism on basal ganglia, and thus evaluate the suggested benefits of coffee consumption in PD.

Material and method: The involvement of A2ARs in the pathology and treatment of PD has been analyzed based on the findings of many published studies examining the effects of A2AR modulation.

Results: Blockage of A2ARs enhances the action of dopamine via D2 receptors on striatopallidal neurons, decreasing their hyperactivity, and exerts a neuroprotective effect, suppressing the neuroinflammation.

Conclusions: Istradefylline, being the only approved A2AR antagonist, was able to reduce the total cumulative dose of levodopa, improve motor control, alleviate postural abnormalities, and provide a reduction in daily 'off' time experienced by patients. Recent findings suggest the effects of drinking one cup of coffee are comparable with ones obtained by the newly introduced medication, presumably via a shared action mechanism by A2AR inhibition.

Keywords: Parkinson's disease, motor function, adenosine receptor, dopamine, striatum

Introduction

Adenosine 2A receptor (A2AR) is commonly known as an acting site of caffeine, which is its nonselective inhibitor, antagonizing both A2ARs and adenosine A1 receptors (A1Rs) [83]. A2AR is involved in many biological functions, including regulation of a cardiac rhythm, cerebral flow, sleep cycle, and response to inflammation. It has also been implicated in the pathophysiology of various neurodegenerative disorders, such as Parkinson's disease (PD) [14]. Since the beneficial effects of coffee consumption have been observed for years, further studies on A2AR modulation were conducted, analyzing the implications in various diseases [52]. Current findings suggest that blockage of A2AR provides neuroprotection, reducing excitotoxicity and regulating neuroinflammation. Moreover, it has been revealed that antagonism of A2ARs can ameliorate motor performance and alleviate complications of PD when administered with levodopa. Istradefylline, developed by the Japanese group Kyowa Hakko Kirin in 2013, is the first approved medication that works by selectively antagonizing A2ARs [14]. Subsequently, in 2019, it received approval from the United States Food and Drug Administration under the brand name Nourianz®, and it is currently indicated in the treatment of Parkinson's disease as an adjunctive to levodopa/carbidopa therapy in adult patients experiencing 'off' episodes [14]. Although the European Medicines Agency still refuses (May 2024) its authorization, stating insufficient efficiency, the results have demonstrated that istradefylline was able to improve motor function, reduce postural abnormalities, decrease the 'off' episodes and minimize the cumulative dose of levodopa [26]. The ongoing research on A2ARs has revealed many interactions with various neurotransmitter circuits, suggesting the possible application of A2AR antagonists as a new approach to treating various neuropsychiatric diseases [14].

Structure and function of adenosine 2A receptors

Adenosine 2A receptors belong to the guanine nucleotide-binding protein-coupled receptor (GPCR) family that responds to extracellular signals, stimulating the cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) cascade. Similarly to dopamine D1 receptors (D1Rs), A2AR is coupled to stimulatory Gs-Golf proteins, so that its stimulation promotes the cAMP-PKA signaling [29]. The formation of cAMP requires the activity of adenylyl cyclase (AC), which is suppressed by coupled to inhibitory Gi protein, activated dopamine D2 receptor (D2R) [29]. A2ARs structurally and functionally interact with the D2Rs, as both localize at postsynaptic sites of indirect striatal pathway neurons, where they form a heteroreceptor complex with antagonistic interactions [79]. In the resting state, A2A and D2 receptors are tonically stimulated by endogenous neurotransmitters, resulting in low psychomotor activity due to the physiological predominance of adenosine receptors [29]. In the presence of reward-related stimuli, the increase in striatal dopamine (DA) enhances the signaling via D2R, inducing psychomotor activity [29]. During the aversive stimulus, the suppressed release of DA strengthens the signaling via A2AR and reduces the D2R-mediated signals, which is observed as psychomotor arrest [29]. Stimulation of A2AR decreases the binding affinity of D2R to its ligands, preventing the effects of dopamine signaling [29]. Therefore, inhibition of A2AR is thought to enhance the effect of D2-mediated activity, which in Parkinson's disease is significantly suppressed due to dopaminergic denervation [12,92]. Moreover, PET imaging studies of patients with PD revealed that the decrease in D2R number is accompanied by an increase in the density of striatal adenosine 2A receptors, and a higher number of A2AR was found in patients who presented with dyskinesia [100]. The upregulation of A2ARs was also observed in Alzheimer's disease, both in animal models and in the brains of patients [25].

At glutamatergic terminals contacting with spiny projection neurons of a direct striatal pathway, presynaptic A2ARs can be distinguished, forming heterodimers with inhibitory A1Rs [18,99]. In these synapses, at basal conditions, presynaptic A1Rs mediate the inhibition of glutamate release. Upon stimulation with higher frequencies, together with abnormal release of ATP, A2ARs become engaged in the adenosinergic transmission, mediating plastic changes of synaptic efficiency [20]. In contrast to A1Rs, activated A2ARs facilitate the glutamatergic transmission and activation of NMDA receptors, leading to long-term potentiation (LTP) [20].

Furthermore, the supramaximal activation of A1Rs can induce the blockage of synaptic transmission, however, the A2AR-mediated generation of LTP can suppress the A1R signaling [20]. During the synaptic potentiation, the neighboring synapses undergo heterosynaptic depression by enhanced inhibitory signaling via A1Rs [20]. A high level of potentiated synapses results in the activation of astrocytic syncytium, evoking the release of extracellular ATP, which is enzymatically degraded into adenosine [20]. Subsequently, adenosine via A1Rs decreases the transmission of neighboring synapses, showing no A2AR activity [20]. In the neurons of the indirect striatal pathway, located at postsynaptic sites A2ARs form heteromers not only with D2Rs but also with cannabinoid and metabotropic glutamate receptors [30,96]. Therefore, the activity of adenosine 2A receptors may affect the transmission of various neurotransmitters, and similarly altered transmission within A2AR functioning network might induce changes in A2AR signaling.

Striatal activity upon dopamine depletion

Parkinson's disease is caused by progressive degeneration of dopamine-secreting neurons in the pars compacta of substantia nigra (SNpc). Since these cells project many axons to the striatum, the denervation of the nigrostriatal pathway occurs, resulting in a reduction of dopamine in this region [121]. Striatum is a cluster of neurons, and being the main nucleus of basal ganglia, it combines the approaching signals to further control the movement [121]. Therefore, due to dopamine depletion, its function is vastly impaired, and the disease progression is linked to the number of damaged dopaminergic neurons, causing the appearance and later worsening of motor symptoms [60]. Approximately 95% of neurons within the striatum represent a type of GABAergic (GABA-gamma-aminobutyric acid) inhibitory cells called medium spiny neurons (MSNs), also known as spiny projection neurons (SPNs). These neurons can be grouped into the neurons of the direct pathway SPNs (dSPNs, striatonigral), projecting directly to the internal segment of the globus pallidus (GPi) and pars reticulata of substantia nigra (SNpr), and ones belonging to the indirect striatal pathway (iSPNs, striatopallidal), that project to the external segment of the globus pallidus (GPe), being indirectly connected to the output nuclei [131].

Spiny projection neurons are accompanied by many interneurons, which based on their electrophysiological activity can be divided into two main groups - tonically active neurons (TANs), which mostly represent cholinergic interneurons (CINs), and the fast-spiking interneurons (FSIs), which are presumably parvalbumin (PV) expressing GABAergic interneurons [115]. Although cholinergic interneurons (CINs) represent only 2% of striatal somata, they significantly regulate activity within this region due to their distinct morphological structure, which enables a single neuron to release acetylcholine (ACh) across a large volume, contacting neurons of the two main striatal pathways [65]. The findings from studies on primates indicate that CINs respond to motivationally relevant stimuli with a short pause in their tonic firing, which is synchronized throughout TANs so that it can efficiently transform into a general reduction of the striatal ACh level [57]. In PD, the ability of these

neurons to induce this pause is lost and linked to excessive striatal cholinergic activity. Recent findings suggest that striatal CINs, being the main source of ACh in the striatum and projecting to both SPN subtypes, modulate the signaling of D2/A2A heteromers by setting appropriate cholinergic tone [119].

Both SPNs and striatal interneurons receive afferent glutamatergic connections from the cerebral cortex and thalamic nuclei as well as the dopaminergic projections originating from the brainstem (SNpc, VTA) [124]. When glutaminergic signals from the motor cortex reach the striatum, dSPNs become activated and send GABAergic signals to the GPi/SNpr, suppressing their tonic inhibitory signals to the ventrolateral nucleus of the thalamus. Once the thalamus is disinhibited, it sends excitatory glutamatergic signals to the motor cortex, initiating the desired movement (Fig.1). At the same time neurons of the indirect striatal pathway receive glutamatergic cortical stimulation via NMDARs and once activated, they send inhibitory signals to the GPe. As the activity of the GPe is suppressed, its tonic GABAergic signals to the subthalamic nucleus (STN) are attenuated, causing its activation. Therefore, STN enhances the GPi/SNpr activity via glutamatergic signals, resulting in increased GABAergic signals to the ventrolateral thalamic nucleus [86,118]. STN plays a key role in preventing the activation of motor cortex regions that are not involved in the initiated voluntary movement [86,118].

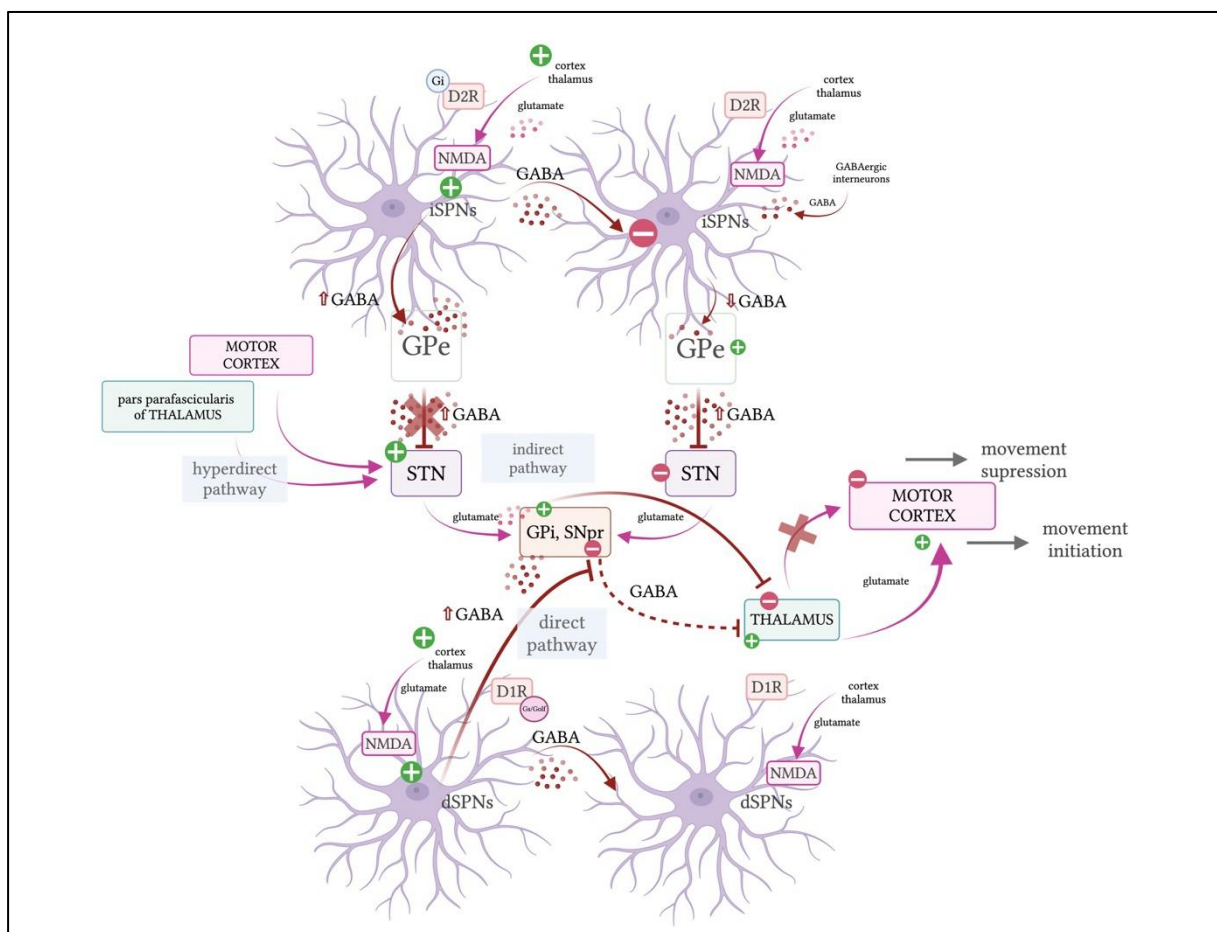


Fig. 1. Overview of movement initiation and suppression via direct and indirect pathways. Created with BioRender.com, (dSPNs-direct spiny projection neurons, iSPNs- indirect spiny projection neurons, GPi - globus pallidus internal, SNpr - substantia nigra pars reticulata, GPe - globus pallidus external, D1/2R - dopamine receptors, STN – subthalamic nucleus, GABA- gamma-aminobutyric acid).

In PD, the loss of dopaminergic innervation increases the excitability of iSPNs' and enhances their sensitivity to cortical glutamatergic excitation due to the absence of D2 receptor-mediated inhibition (Fig.2). At the same time low DA levels decrease the excitability of D1-SPNs, suppressing their function of movement initiation via diminished GABAergic signals to GPi/SNpr. Subsequently, the indirect striatal pathway becomes excessively active resulting in abnormal activity of STN [85,121]. Since D2Rs display a higher affinity for dopamine than D1Rs, iSPNs maintain the dopaminergic tone during low DA levels. In PD this dopaminergic signaling is outbalanced by upregulated signaling via A2ARs leading to hyperactivity of iSPNs [85,121]. Striatopallidal neurons prevent the initiation of motor programs that compete with ones simultaneously initiated by D1-dSPNs. Therefore, A2AR upregulation is believed to disrupt the static component of motion, including adequate muscle tone and posture via increasing excitability of iSPNs [85,121].

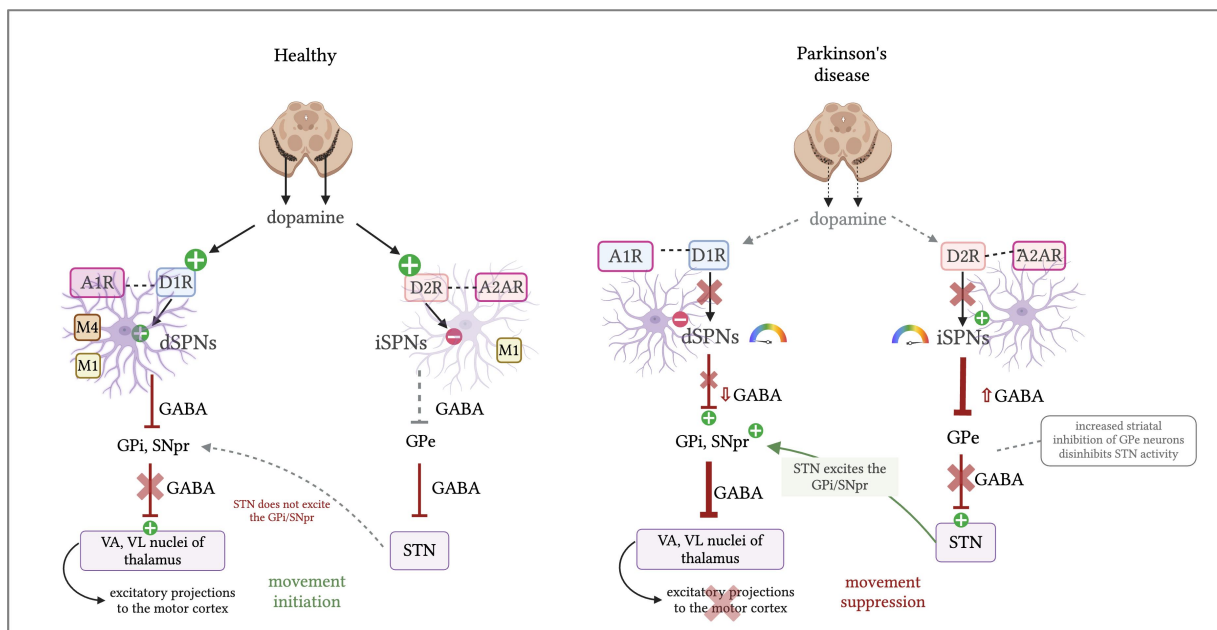


Fig. 2. Overview of striatal direct (dSPNs) and indirect spiny projection neurons (iSPNs) pathways in healthy individuals and during Parkinson's disease. Created with BioRender.com, (GPi - globus pallidus internal, SNpr - substantia nigra pars reticulata, GPe - globus pallidus external, D1/2R - dopamine receptors, A1R/A2AR - adenosine receptors, dSPNs - direct spiny projection neurons, iSPNs - indirect spiny projection neurons, STN - subthalamic nucleus, GABA- gamma-aminobutyric acid, VA - ventral anterior, VT- ventrolateral).

Signaling via subthalamic nucleus

The hyperactive neurons of the indirect pathway via increased release of GABA onto GPe, and the attenuated GABAergic transmission within the GPe-STN connection, result in the disinhibition of the subthalamic nucleus. Subsequently, the abnormal activity of STN initiates excitatory 'NoGo' signals to GPi, which begin to outweigh the activity of the direct 'Go' pathway, causing the reduction in movement and development of parkinsonian symptoms such as bradykinesia [85]. The neurons of STN have intrinsic membrane properties, which cause the generation of spontaneous rhythmic firing patterns without the presence of input. The activation state of STN not only results from its autonomous activity, but also from the direct glutamatergic excitatory afferents arising from the motor cortex, and from the parafascicular (PF) thalamic nucleus, establishing the hyperdirect pathway [16]. Autonomously active STN by interacting with approaching external signals, generates its total output. Moreover, neurons of the STN send axon collaterals to the internal as well as the external part of the GP, and these neurons from GPe innervate the same group of GPi neurons

that the axons from STN [138]. Studies exhibited that during deep sleep, the cortical glutamatergic inputs control STN neuron firing in rhythmic bursts of action potentials of approximately 1Hz [16]. The precise level and pattern of cortical excitation of STN neurons alongside their inhibition by GPe are essential to maintain physiological motor activity, however found significantly disrupted in Parkinson's disease [16].

Baudrexel et al., (2011) have observed that patients with PD during rest exhibited increased activity in STN–motor cortex connection compared to healthy controls and suggested that the hyperdirect pathway may be highly involved in the pathophysiology of PD [137]. The hyperdirect cortical excitatory inputs and/or increased inhibition of GPe via iSPNs' activity cause the subthalamic neurons to fire repetitively at much higher frequencies, contributing to basal ganglia activity. It has been suggested that GABAergic inputs originating from GPe play a crucial role in resetting the oscillatory cycle of ionic mechanisms that govern the rhythmic firing of STN neurons [137]. Therefore, the loss of D2 stimulation of iSPNs with upregulated A2AR signaling would facilitate the occurrence of STN's burst firing of action potentials via GABA-mediated inhibitory postsynaptic potentials [16]. The findings of Chu et al. (2020) demonstrated that after DA depletion, the inhibitory GPe-STN synaptic connection is abnormally strengthened and the number of release sites per GPe-STN axon terminal is increased [16]. Simultaneously, animal models of PD exhibit a vast decrease in the number of cortico-STN synaptic terminals with decreased strength of synaptic connection [16,17]. This pathological GPe-STN strengthening is thought to be a result of the heterosynaptic long-term potentiation induced by originated from motor cortex, glutamatergic inputs to STN. In PD, the reduced GPe-STN GABAergic transmission becomes offset in phase to cortical STN excitation resulting in the hyperactivity of subthalamic NMDARs, followed by a compensatory increase in strength of GPe-STN synapses [71]. The intrinsic mechanism of STN neurons balances the cortical excitation with adequate GABAergic inhibition from GPe, causing hyperpolarization of membrane potential, producing a pause, and resetting the phase of autonomous action [71]. However, if the cortical excitatory stimulus happens offset in phase, GPe promotes the cortical patterning through both disinhibition and increased availability of postsynaptic Na⁺ and Ca²⁺ channels post-inhibition. In PD the activity of STN occurs off-phase to GPe activity and in-phase to cortical activity, suggesting that inhibitory inputs from GPe are less proficient in suppressing cortical excitation via shunting but more adept at facilitating the cortical modulation of STN activity by disinhibiting postsynaptic voltage-gated Na⁺ and Ca²⁺ channels involved in the generation of STN rhythmic activity [6,16]. Therefore, the overly active hyperdirect pathway in PD is believed to be a result of incoordination between inhibitory signals of GPe and excitation mediated by cortical inputs, rather than caused directly by enhanced transmission of cortico-STN connection [16]. This extrinsic synaptic excitation of STN neurons does not generate their firing pattern, however, controls the timing of action potentials created intrinsically [139].

The hyperdirect and indirect striatal pathways, transmitting cortical excitatory inputs to STN are thought to be the origin of Parkinsonian beta oscillations, which are presumably generated within the GPe-STN connection [3]. Moreover, the atypical neuronal activity in the cortico-basal ganglia-thalamocortical network of PD patients was linked to motor symptoms, including akinesia, bradykinesia, and rigidity [16,71,72]. The appearance of synchronized neuronal beta oscillations of approximately 10-30Hz and the unsuccessful attempts in generating 30-60Hz gamma-band oscillations before and during movement are thought to be important in the pathophysiology of Parkinsonian symptoms [121]. The loss of dopaminergic modulation is followed by the downregulation of autonomous activity in the motor area of the

STN, as observed in toxin models of PD. Subthalamic nuclei of Parkinsonian 6-OHDA-treated mice exhibited a decrease in frequency, regularity, and incidence of spontaneous firing [16]. McIver et al. suggest the loss of autonomous STN activity in Parkinsonian models is a result of reduced GPe activity due to the enhanced release of GABA by hyperactive iSPNs [78].

The suppression of corticostriatal transmission on iSPNs requires the activation of postsynaptic D2 and mGluR5 receptors. Thus, in PD long-term depression (LTD) cannot be induced due to the absence of D2-signaling in iSPNs. Therefore, iSPNs present with long-term potentiation (LTP) which is mediated by A2AR and NMDA receptors and results in the persistent strengthening of synapses, facilitating a sustained elevation in synaptic transmission within a neuronal network [16].

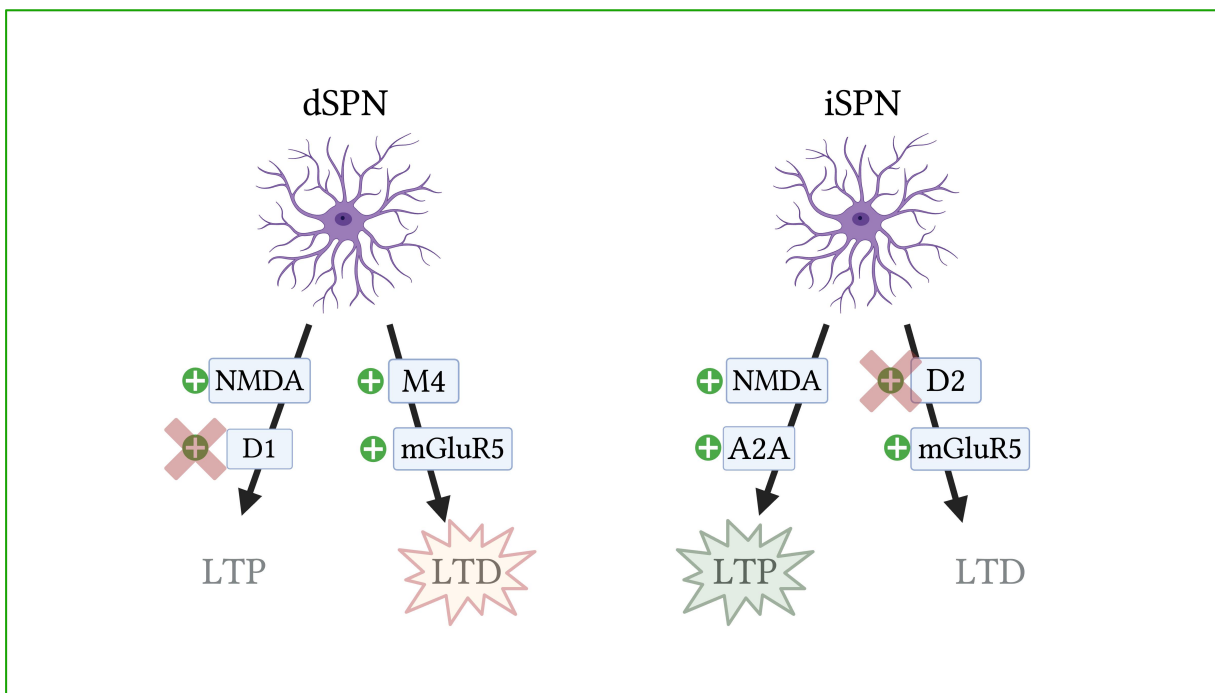


Fig. 3. Receptors affecting long-term potentiation (LTP) and long-term depression (LTD) of striatal direct (dSPNs) and indirect spiny projection neurons (iSPNs). Created with BioRender.com, (NMDA- N-methyl-D-aspartate, M4- muscarinic, D1/2-dopaminergic, A2A- adenosine 2A, mGluR5- type 5 metabotropic glutamate receptors).

Regulation of striatal activity via interneurons

The associated with motor deficits parkinsonian oscillations, representing the increased striatal spontaneous activity and neural synchrony, are also the result of tonically active neurons (TANs), mainly cholinergic (CINs), which regulate striatal activity by releasing acetylcholine [91]. During CINs' tonic firing, their activity determines the baseline level of striatal DA via stimulation of nicotinic acetylcholine receptor (nAChR) located on dopaminergic axon terminals by the released ACh [56]. This results in local depolarization and induction of DA release that occurs regardless of the action potentials evoked at DA neuron soma [65]. Kondabolu et al. (2016) observed the optogenetic blockage of these neurons leads to a transient decrease of oscillations together with alleviation of motor symptoms [57,76] Findings from studies on primates indicate that CINs respond to associated with evoked DA release motivationally relevant stimuli, with a short pause in their tonic firing, which is synchronized throughout TANs so that it can efficiently transform into general reduction of striatal ACh level [57]. In PD, the ability of cholinergic neurons to induce this

pause is lost, enhancing the cholinergic transmission in the striatum. CINs, being the main source of ACh in the region, projecting to both SPN subtypes, are thought to modulate the signaling of D2/A2A heteromers by setting appropriate cholinergic tone. It is also suggested that CINs play a crucial role in the development of levodopa-induced dyskinesia (LID) as ablation of these cells significantly reduced LID in mice PD models, with sustained therapeutic action of L-DOPA [128].

The glutamatergic inputs received by CINs primarily originate from the thalamus [24]. According to Kim et al., the excitatory stimulus produces an initial increase in the TAN firing by depolarization of their membrane and subsequent influx of Ca²⁺ ions via L-type voltage-gated calcium channels (VGCCs, Cav1). The withdrawal of these inputs underlines the pauses of CIN firing, during which ACh release is suppressed allowing the phasic release of DA. Thus, the duration of this pause depends on the current level of dopamine [56]. Termination of excitatory stimulus causes the efflux of potassium ions via calcium-activated K⁺ channels, resulting in hyperpolarization and the occurrence of slow after-hyperpolarization current (sAHP), that is responsible for slow rhythmic bursting [56]. Subsequently, the inward DA-dependent h-current (I_h) is induced, increasing the membrane potential [56]. Zhang, Reynolds, and Cragg, (2018) have shown that pauses in CIN firing reflect the withdrawal of excitatory input caused by a delayed rectifier potassium current (IKr) and local modulation via D2R-mediated DA signaling [133]. Cholinergic interneurons express high levels of the D2Rs, which suppress the release of ACh by preventing the opening of Cav2 Ca²⁺ channels in response to membrane depolarization. D2Rs occasionally slow the CIN autonomous pacemaking, reducing spiking at terminal release sites [56]. Postsynaptic D2Rs of CINs suppress the autonomous firing via voltage-sensitive sodium channels or hyperpolarization-activated cyclic nucleotide-gated (HCN) currents [1]. Dopamine affects CINs' refractory period, reducing their activity by downregulating the h-current upon D2R stimulation [56]. Brief elevations in striatal DA level reduce the release of ACh, however, the abolished D2R stimulation in PD is unlikely to control the basal cholinergic tone. Consistently, no difference in the rate of CINs' autonomous spiking was observed after dopamine depletion. D2 current is not sufficient to potentiate the coincident CIN pause, which is instead dominated by the faster IKr current [56,133].

Acetylcholine can both stimulate and modulate dopamine release via activation of nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors, respectively [65]. In striatum mAChRs can be found on SPNs, interneurons and glutamic axon terminals [64]. M1 mAChRs, coupled to G_{αq} are expressed postsynaptically on both direct and indirect SPNs, whereas G_{αi}-coupled M4 mAChRs preferentially localize on D1-expressing dSPNs [64]. M4 and M2 mAChRs, expressed on striatal CINs act as autoreceptors (AR), suppressing the enhanced cholinergic transmission, and further decreasing the level of striatal dopamine [65].

Goldberg, (2005) reported that sAHP, responsible for slow rhythmic bursting is triggered by calcium influx via Cav1 channels. However, Cav1 currents are not affected by either muscarinic agonists or by D2R activation or inhibition [34]. Interestingly, Hernández-González et al. (2013) reported that the Cav1 currents are enhanced by A2ARs, promoting long hyperpolarization and rhythmic bursting. Thus, again the effects induced via D2R signaling are counteracted by adenosinergic stimulation [41]. Moreover, adenosine has been reported to reverse the N-type Cav2 currents in both CINs and the two SPN subpopulations, through membrane G-protein pathways [1]. Kurokawa et al., (1994) were the first to reveal that A2AR stimulation increases the release of ACh from striatal nerve terminals [62]. Striatal

cholinergic interneurons express both adenosine type 1 and 2A receptors, where Preston et al. found that tonic signaling of endogenous adenosine is linked to the enhanced release of ACh [97]. The blockage of A2AR was reported to counteract this activity. Moreover, they have observed that a selective A2AR antagonist (CSC) was able to reverse the increase in ACh induced by the A2AR agonist CGS21680 [97]. In contrast, the combination of opioid and GABA receptor antagonists could not repeat the effect of CSC, implying that A2AR was not acting presynaptically on GABAergic striatopallidal recurrent collateral nerve terminals [97]. Because of the inability of these receptor antagonists to follow the CSC-induced effect on the potassium-evoked release of ACh, it is supposed that the endogenous adenosine tone is also directly exerted on the cholinergic interneurons [97].

Tozzi et al. revealed the D2 and A2A receptors in physiological conditions display synergistic action that results in the inhibition of CINs' firing rate and presumably to a reduction of striatal ACh, which in turn may influence the synaptic activity of both direct and indirect SPNs via endocannabinoid signaling [120]. They further report, that the effects of D2/A2A modulation of glutamate release, as well as the D2R-mediated SPNs' activity, are diminished in the presence of an M1R inhibitor, as observed in PD models. Because M1Rs are expressed in both subpopulations of SPNs, the suppression of cholinergic transmission upon M1 blockage may modulate the glutamatergic transmission at synapses of both dSPNs and iSPNs [120]. Consistently, Kharkwal et al. have observed the cataleptic effect of D2 antagonists was not induced by atypical antipsychotic medications which exhibited additional M1R antagonism. Blockage of D2Rs suppresses the D2R-mediated inhibitory signaling in iSPNs causing their activation, and the antagonism of D2R on CINs increases their activity with the subsequent release of ACh. Acetylcholine, acting on iSPNs' M1 receptors further enhances the activity of indirect pathway [32]. Thus, catalepsy that results from D2R antagonism depends on the blockade of D2Rs on cholinergic interneurons, where it induces CIN activation, followed by enhanced ACh signaling onto striatopallidal neurons [32]. According to Tozzi et al., (2011), blockage of M1R suppresses the D2R/A2AR-mediated modulation of excitatory signals in both SPN subpopulations [119].

In addition to D2Rs, the interaction between ACh and DA is also mediated by D1/5 receptors which are expressed in CINs' dendrites [1]. It is thought that activation of D1/D5Rs enhances the excitability of CINs through a cAMP-dependent mechanism, that results in the closure of K⁺ channels and induces the opening of nonselective cation channels [1]. Furthermore, activation of M4Rs expressed on dSPNs results in the increase of SPNs excitability via enhancing the CaV1 channels, which were also reported to be upregulated by A2ARs [1].

Enhanced expression of regulators of G-protein signals in PD

The modulation of CIN activity by presynaptic D2Rs via voltage-gated Cav2 channels is further controlled by regulators of G-protein signals (RGS) [1]. According to Ding et al. (2006), the increased cholinergic tone in PD may underline the desensitization of M4 autoreceptors, rather than the defective D2 signaling. They have revealed that upon dopamine depletion, the D2R-mediated signaling in CINs is not altered but rather suppresses the function of M4 autoreceptors (M4-ARs) [23]. In control conditions, M4-ARs regulate the autonomous CIN activity and ACh release by opening the Cav2 channels. However, upon DA depletion, the function of M4Rs is disrupted by upregulated expression of RGS4, which in PD causes the disinhibition of N- and P-type Cav2 channel currents and ACh release [23]. Ding et al. (2006) state the general expression of RGS4 mRNA in a DA-depleted environment is decreased, however, in cholinergic interneurons, its expression is highly upregulated [23]. In

cholinergic interneurons, stimulated D2Rs reduce the Cav2 channel currents via a pathway controlled by a regulator of G protein signaling 9 (RGS9), causing the decrease of ACh [114]. In caudate and putamen from patients PD, there also has been found an increased expression of RGS9, which attenuates an endogenous D2R-mediated inhibition of Cav2.2 Ca²⁺ channels, causing the influx of Ca²⁺ ions into CINs. Abudukeyoumu et al. (2018) reported that upon dopamine depletion, Cav2 channel opening of M4 autoreceptors and pacemaking are attenuated by increased expression of RGS9 [1,114]. Moreover, the observed increase of RGS9 negatively correlated with the level of dopamine, its metabolites, and transporters in the putamen. Consistently, its concentration and mRNA decrease in dopamine-depleted animals after treatment with levodopa [1]. Induction of LTD in iSPNs depends upon postsynaptic D2R activation, which diminishes mediated by RGS4 inhibition of mGluR5-dependent endocannabinoid production [67]. More recently, postsynaptic M4R signaling in dSPNs was found to promote LTD induction through suppression of RGS4 – establishing a clear mechanistic parallel to the situation in iSPNs [67,109].

In addition, D2Rs stimulate phospholipase C (PLC) and protein kinase C (PKC) through a non-canonical pathway to promote phosphorylation and inactivation of voltage-dependent Nav1 Na⁺ channels, thus slowing or stopping the ongoing pacemaking [13,77]. Activated D2Rs also reduce the amplitude of HCN currents driving pacemaking, presumably by reducing cAMP, which allosterically enhances HCN gating.

Retrograde endocannabinoid signaling

The postsynaptic A2ARs of iSPNs form heteromers not only with the D2Rs but also with the cannabinoid 1 receptors (CB1R). Moreover, these receptors are thought to act within A2AR–CB1R–D2R heteromers, as suggested by the findings of animal striatal slices. CB1R is the acting site for the main active ingredient of marijuana Δ^9 -tetrahydrocannabinol (THC), and for produced within body tissues endogenous cannabinoids, such as anandamide and 2-arachidonoyl glycerol (2-AG). Martire et al. (2010) reported the existence of presynaptic interactions between A2A and CB1 receptors in the striatum, and the presynaptic CB1Rs were revealed on both terminals and collaterals of SPNs [63, 74, 84]. Since presynaptic A2ARs are mainly located in cortical glutamatergic terminals projecting onto neurons of the direct pathway, the presynaptic A2A/CB1 interaction is thought to occur in D1-dSPNs, which selectively express M4Rs [74]. These A2AR–CB1R–D2R heteromers were found to be disrupted upon the treatment with levodopa, and the diminished interactions within the receptor complex were suggested to be involved in the development of LID [140]. Moreover, dyskinesia in PD was linked to the upregulation of CB1R-CB2R in activated microglia [8].

The presynaptic A2A–CB1 receptor complex in corticostriatal terminals of dSPNs is thought to modulate the signals between the cortex and striatum. Tozzi et al. reported that under physiological conditions, simultaneous D2Rs activation with the blockade of A2ARs reduces the striatal glutamatergic signals via presynaptic mechanism not only within the indirect pathway but on a larger scale [119]. According to Kreitzer and Malenka (2007), the presynaptic effect mediated by eCBs released from postsynaptic neurons is responsible for the suppression of iSPNs' activity via D2R stimulation [61]. Activation of D2Rs on iSPNs is followed by the production and release of eCBs, which by stimulating located in glutamatergic terminals CB1Rs, mediate the inhibition of striatal glutamate release, which results in further decrease of stimulatory signals to iSPNs [29]. Activation of CB1R results in an inhibition of adenylyl cyclase (AC) and N-type voltage-dependent Ca²⁺ channels, reducing the cAMP-mediated signaling [74,117]. Stimulation of A2AR within the

antagonistic A2A-D2 heteromer in iSPNs prevents the D2R-mediated inhibition of excitatory glutamatergic transmission, which results in the activation of NMDARs and the increase in permeability to calcium ions [117]. Enhanced signaling via A2AR leads to the hyperactive state of iSPNs, eventually reducing the endocannabinoid signaling [117]. Consistently, inhibition of A2ARs is thought to stimulate endocannabinoid signaling, inducing LTD and decreasing the excessive activity of iSPNs [30].

It was observed that A2AR agonists inhibit the synaptic effects of CB1 stimulation, reducing the CB1R-mediated inhibition of glutamate release. However, a basal level of A2AR activity is required to enhance the CB1-mediated phosphorylation of DARPP-32 and to induce motor effects by CB1 agonists [116]. It was observed that the effects induced by CB1 agonists depended on the A2AR coactivation in striatal slices. These A2ARs are thought to display a permissive role, allowing the CB1 signaling via A2A-CB1 receptor complexes. The decrease in glutamatergic corticostriatal signaling induced by CB1R agonists was suppressed by A2AR antagonists as well as by genetic knockdown of striatal postsynaptic A2ARs. The findings suggest that A2AR antagonism displays a similar effect as antagonism of CB1R, consistently, the pharmacological and genetic blockage of A2ARs resulted in a reduction of motor depression and cataleptic and rewarding effects of CB1 agonists [30]. Lerner et al. (2010) demonstrated that inhibition of A2ARs potentiates the release of eCBs, inducing LTD at corticostriatal synapses of iSPNs, whereas not of dSPNs [66]. It is believed that A2ARs regulate the CB1 signaling via modulation of mGlu5Rs, which are known to induce the release of endocannabinoids [116].

The striatal endocannabinoid signaling is also regulated by acetylcholine released by cholinergic interneurons. Stimulation of M1Rs at inhibitory synapses promotes the production of eCBs, followed by retrograde CB1R activation, that suppresses the inhibitory synaptic transmission. However, at excitatory glutamatergic synapses agonists of M1Rs were reported to reduce the postsynaptic Cav1.3 currents, which, in turn, suppress the endocannabinoid generation and so the stimulation of presynaptic CB1R [1]. In dystonia, upon stimulation, D2Rs present with excessive inhibition of Cav2.2N-type currents. Mice that were genetically deprived of the dystonia 1 protein, responded to activation of thalamostriatal inputs with a brief pause and increased rebound activity in CINs that could result from a postsynaptic increase and a presynaptic decrease in M1 and M2-dependent currents.

It is thought that the A2AR-mediated regulation of glutamate release to some extent depends on its heteromerization with A1 receptors. According to Ciruela et al., (2006,) A2AR activation reduces the A1R-mediated inhibition of glutamate release [18]. Chen et al., (2012) revealed that neurabin, which is a neural tissue-specific protein, suppresses the signaling via A1R by assembling a complex with RGS4, a protein-reducing G-protein signaling [15]. A1R is coupled to Gi/Go proteins, and its stimulations result in an increase of PLC and K⁺ ions, whereas decreases adenylate cyclase (AC) and calcium ions.

A proper A2A/D2 activity mediates the downstream pathways involved in the regulation of endogenous endocannabinoid (eCBs) production, such as PKA/cAMP and RGS4, that mediate the mGluR5-induced eCBs signaling [75]. Inhibition of RGS4 was reported to restore the eCBs-mediated LTD during both D2R antagonism and dopaminergic denervation. Lerner and Kreitzer (2012) reported that in iSPNs, RGS4 connects the cAMP/PKA pathway with endocannabinoid signaling with the induction of LTD [67]. They suggest that the enhanced activity of PKA upon A2AR stimulation results in the upregulation of RGS4, thus inhibiting

the mGluR-Gq function and suppressing the endocannabinoid signaling [67]. Lerner and Kreitzer imply that GTPase-accelerating protein RGS4 is required to induce the effect of D2 and A2A receptors on eCBs-mediated LTD, mediating dopamine signaling, synaptic plasticity, and motor behavior, and maybe a promising non-dopaminergic target for modulating basal ganglia circuitry [67]. RGS4 is phosphorylated by PKA and inhibits mGluR-Gq signaling [67].

Adenosine A2AR signaling - summary

Current evidence indicates that the commonly known A2AR-mediated cAMP-PKA signaling pathway is not the only one induced upon stimulation of this receptor. It has been revealed that activation of A2AR induces signaling through various pathways, that include mitogen-activated protein kinases (MAPK), such as ERK1/2 (extracellular signal-regulated kinases), c-Jun N-terminal kinases (JNKs) or p38, phosphoinositide 3-kinases (PI-3K), protein phosphatases, protein kinase C (PKC), Src kinases, or peroxisome proliferator-activated receptor (PPARs) [20]. The activity of A2ARs affects the downstream signaling of many receptors of various neurotransmitters.

In the striatal neurons as well as in glutamatergic nerve terminals in the striatum, A2ARs colocalize with metabotropic glutamate receptors 5 (mGlu5R), which play a crucial role in the regulation of the cAMP/PKA pathway, mainly in iSPNs [21,108]. These excitatory receptors, coupled to Gq-protein, mediate the signaling via protein kinase C (PKC), and upon receiving stimulus, they induce the signaling via activated phospholipase C (PLC), which evokes the release of intracellularly stored Ca²⁺ ions [21]. It is thought that mGlu5Rs function within the receptor complex with A2ARs, displaying synergistic interactions [108]. Simultaneous activation of these two receptors significantly increases the release of GABA from ventral striatopallidal neurons, and together they induce downstream responses, including the increase in c-fos expression and phosphorylation of DARPP-32 (dopamine, cAMP-regulated phosphoprotein of 32,000 kDa). Moreover, both receptors potentiate the transmission via NMDAR (N-methyl-D-aspartate receptor) [21,82,108].

Examining rat hippocampus, Krania et al., (2018) have observed that A2ARs are required to induce the mGlu5R- and D1R- mediated phosphorylation of ERK1/2 [59]. Moreover, A2ARs were reported to display a permissive role for cannabinoid 1 receptors (CB1) as their presence is required to enhance the CB1-mediated phosphorylation of DARPP-32 and to induce motor effects by CB1 agonists [116]. It is thought that A2ARs regulate the CB1 signaling via modulation of mGlu5Rs, which are known to induce the release of endocannabinoids [116]. Activated upon A2AR stimulation, protein kinase A, catalyzes phosphorylation of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein) at Thr34, converting DARPP-32 into an inhibitor of protein phosphatase-1 (PP-1). However, in order to induce the phosphorylation of AMPAR subunit GluA1, A2AR stimulation requires concomitant signaling via activated mGlu5Rs in iSPNs [21,107]. Findings of Dell'Anno, Pallottino and Fisone, (2013) imply the activation of striatal mGlu5Rs induces cAMP/PKA/DARPP-32-dependent phosphorylation of AMPAR subunit GluA1 at Ser845, which occurs selectively in iSPNs, and it is exerted via potentiation of A2AR tonic signaling [21]. This pathway, as they report can be prevented by activation of dopamine D2 receptors, which are coupled to inhibitory G proteins that inhibit AC [21]. While A2AR stimulation increases the phosphorylation at Thr34-DARPP-32 and reduces the phosphorylation at Thr75-DARPP-32, the antagonism of A2AR mediated by caffeine increases the phosphorylation state of DARPP-32 at Thr75 [42,69,107]. Lindskog et al., (2002) have observed the enhanced phosphorylation of Thr75-DARPP-32 mediated by

both A2AR antagonist SCH 58261 and caffeine, was remarkably diminished in mice that were genetically deprived of DARPP-32 [69]. Moreover, these mice did not display the increase in locomotion, that was observed in DARPP-32 intact mice. A2ARs increase, whereas D2Rs decrease the levels of cAMP, and activation of these receptors, respectively, enhances and suppresses the cAMP/PKA mediated signaling. The stimulation by adenosine provides a basal tonic activity of the cAMP/PKA/Thr34-DARPP-32 pathway, that is required to induce the action of stimulated or inhibited receptors [107]. According to Lindskog et al., induction of stimulatory effects on motor activity via caffeine and SCH 58261 requires the presence of DARPP-32, and it is suggested that the blockade of a tonically active A2A/PKA/PP-2A/Thr75-DARPP-32 pathway is crucial in stimulatory actions of caffeine [69,107,111]. Furthermore, the fact that dyskinetic animals displayed abnormally high levels of phospho-Thr34 DARPP-32 supports the idea that A2AR upregulation is involved in the development of LID [107]. Even though both D1R and A2AR induce the cAMP/PKA cascade and enhance the phosphorylation at Thr34, the effects of mGlu5R stimulation are mediated via activated by endogenous adenosine via A2ARs, but not D1Rs [107]. This may underline the additional effects provided by A2AR antagonists to standard dopaminergic therapy. The finding suggests that antagonism of A2AR antagonists counteracts the increase in Thr34-DARPP-32 phosphorylation that was observed following treatment with selective D2 receptor antagonists. Likewise, the ability of D2 antagonists to increase Thr34-DARPP-32 phosphorylation was dramatically reduced in A2A receptor KO mice [107].

α -synuclein-induced neuroinflammation

α -synuclein (α -syn) is the main component of Lewy bodies, the insoluble cytoplasmic protein inclusions found in SN of patients with PD. Recent findings suggest that its soluble prefibrillar forms (soluble oligomers) are mainly involved in the induction of the neurodegenerative process [104]. Changes in the extracellular matrix or cellular damage send signals that stimulate the function of microglia, the immune cells of the central nervous system (CNS). Consistently, activation of microglia has been found in the striatum and SN of patients during PD [104]. Upon activation, these cells present with phagocytic activity [140]. Normally, microglia maintain brain homeostasis and generate ATP via oxidative phosphorylation, whereas in response to inflammatory stimulation their metabolism shifts to aerobic glycolysis. In response to α -synuclein activation of microglia results in the induction of a neuroinflammatory process that is linked to neurotoxicity [104]. In PD, the α -synuclein aggregates enhance the glutamatergic transmission, exacerbating glutamate-related excitotoxicity. Moreover, the activation of glutamate receptors on microglia increases the release of proinflammatory factors, amplifying the occurring neuroinflammatory process. Li et al. have reported that the α -synuclein deposits, by interacting with toll-like receptors (TLRs), promote activation of the microglia NLRP3 inflammasome, inducing the neuroinflammatory process [68]. This is followed by the translocation of nuclear factor kappa-B (NF- κ B) and the release of proinflammatory mediators that further lead to the damage of dopaminergic neurons [68].

The presence of α -synuclein leads to the impairment of mitochondrial function. The internalization of its fibrils by microglia induces the excessive production of mitochondrial reactive oxygen species (mtROS) that disrupt the complex-I-dependent respiration and decrease mitochondrial membrane potential, augmenting inflammatory response and the loss of dopaminergic neurons in PD pathology [68]. mtROS also activate the NLRP3 inflammasome, serving as a second activation signal [68]. Ludtmann et al. (2018) reported that the α -syn oligomers selectively oxidize the β subunit of ATP synthase, causing mitochondrial lipid

peroxidation and increased mitochondrial permeability transition pore opening [150]. ATP is one of the damage-associated molecular patterns (DAMPs), which are released into extracellular space due to cellular stress, necrosis as well as during neurodegeneration [127]. The massive release of ATP occurs through the overly permeable plasma membrane of neural tissue and has been associated with various neurodegenerative diseases, but also with other pathophysiological events including mechanical damage, ischemia, or status epilepticus [47]. He et al., (2017) reported that neuroinflammation and the associated release of ATP induce functional changes in the activity of ATP-gated channel receptor P2X7, which under physiological conditions remains inactive [40]. P2X7 receptor (P2X7R) is mainly expressed in microglia and ATP enables its function [40]. It is thought that P2X7R triggers the influx of Ca²⁺ ions and the formation of ROS, which are responsible for the reduction of mitochondrial respiration and the induction of apoptosis in neuronal cells [126]. He et al., (2017) have revealed that P2X7R is coupled to AKT and ERK pathways, which contribute to the cell death upon P2X7 activation in microglia [40]. The microglia P2X7Rs mediate the shedding of ectosomes, the process mediated by the activity of acid sphingomyelinase and the effector protein p38. This occurs not only in microglia but also in astrocytes which also express P2X7R, however, microglia constitute a significant source of these shed vesicles. The excessive influx of Ca²⁺ ions via P2X7 channels with the depletion of intracellular ions and metabolites results in the necrosis of P2X7-expressing microglia cells [47]. ATP-dependent activation of P2X7Rs induces necrosis by promoting the formation and opening of nonspecific membrane pores, leading to loss of intracellular content and activation of the caspase pathway, causing apoptosis in glial cells.

The exposure of microglia cells to α -synuclein resulted in the activation of their NADPH oxidase which was dependent on the presence of P2X7Rs and further led to the cleavage of caspase-3 in the dopaminergic PD model cell line SH-SY5Y [47]. The oxidative damage by α -synuclein of SH-SY5Y cells was due to P2X7R activation and the simultaneously occurring increased release of ATP as well as its decreased degradation by ecto-ATPase. These α -Synuclein aggregates in addition caused the release of the excitotoxic glutamate from cultured microglial cells. It has been already revealed by Carmo et al., (2014) that the inhibition of P2X7 receptors prevented Parkinsonian neurodegeneration and associated symptoms [11]. Wilkaniec et al. (2020) have observed that the pretreatment with P2X7R antagonist significantly prevented the cell death induced by α -synuclein, as well as the α -syn and ATP-induced generation of free radicals, including mtROS [126].

The role of A2AR during neuroinflammation

Current findings indicate that ATP serves a dual function in neurodegeneration, directly by initiating the inflammatory process via P2X7R activation, and indirectly, via A2ARs upon the extracellular enzymatic conversion to adenosine. Adenosine and ATP modulate the neuroinflammatory reactions, oxidative stress, and cell death through activation of A2A and P2X7 receptors, respectively. Recent findings underline the importance of A2ARs in the pathophysiology of synucleinopathies. In PD, the observed dysregulation of the A2ARs has been linked to the presence of α -synuclein deposits and α -synuclein-mediated neurotoxicity [10]. The 6-OHDA-treated animal Parkinsonian models exhibited increased ATP release from striatal nerve terminals at the prodromal phase of the disease with increasing levels of adenosine. Ena et al., (2013) revealed that striatopallidal neurons express an enzyme, ecto-5' nucleotidase (CD73), which enables iSPNs to generate extracellular adenosine from adenosine monophosphate (AMP) [4,27]. Inhibition of adenosine generation by the blockage of CD73, which facilitates the ATP breakdown, effectively decreased neuroinflammation

orchestrated by microglia, leading to notable improvements in the survival of dopaminergic neurons and motor function in models of PD [36]. It is suggested that the upregulated generation of adenosine reflects the excessive activation of A2ARs, thus inhibition of CD73 might become a new approach to the treatment of PD [101].

The evoked ATP release, followed by a CD73-mediated increase in extracellular adenosine is believed to underline the upregulation of A2ARs observed in PD [151]. Adenosine stimulates the A2ARs localized at postsynaptic sites of iSPNs, and influences corticostriatal long-term potentiation which is thought to be a key pathway during abnormal synaptic plasticity associated with the onset of motor symptoms in PD [36,122,151]. Moreover, Sancho et al. (2022) have revealed that the endogenous adenosine activated ATP-sensitive K⁺ channels in the brain capillary network via the A2AR-Gas-AC-PKA signaling pathway [105]. Upon A2AR activation, PKA phosphorylates sites within the K⁺-ATP channel complex promoting its opening. Subsequently, the efflux of potassium ions causes hyperpolarization of the cell membrane with the closure of voltage-gated Ca²⁺ channels [105]. This leads to vasodilation of brain arterioles and following increase in cerebral blood flow [105].

Stimulation of A2ARs mediates the activation of neurotrophin receptors and enhances their trophic functions on BDNF (brain-derived neurotrophic factor)-mediated synaptic transmission and LTP, as well as on GDNF (glial cell line-derived factor)-mediated action in striatal dopaminergic terminals [83]. The neuronal effects of BDNF are induced via two transmembrane receptors: the p75 neurotrophin receptor (p75NTR) and the TrkB receptor tyrosine kinase, thus BDNF from cortico-striatal afferents modulates their plasticity via TrkB in SPNs. According to Andreska et al. (2023) activation of D1 receptors induces translocation of TrkB to the cell surface, increasing sensitivity for BDNF, however in DA-depleted cultured dSPNs, animal models of PD as well as in postmortem brain of parkinsonian patients, the responsiveness to BDNF was reduced with observed intracellular TrkB clusters [2]. Kang et al. (2017) state that the neurotrophic activity of the TrkB/BDNF signaling pathway in PD is suppressed upon the binding of α -synuclein onto the kinase domain of TrkB. Thus, the endocytosis of TrkB is disrupted, and TrkB protein levels are reduced, whereas its ubiquitination is increased which later causes subsequent dopaminergic neurodegeneration [54]. A2AR which is stimulated by adenosine, functions within the physiological negative feedback mechanism responsible for the limitation and termination of both tissue-specific and systemic inflammatory responses [89]. In addition, A2AR is involved in microglial process retraction, which occurs during prolonged brain injury or disease, upon the previous ATP-driven process extension [90]. These data led the authors to conclude that A2AR activation plays a mandatory role in controlling the release of BDNF from activated microglia, as well as the autocrine/ paracrine proliferative role of BDNF [35]. Lipopolysaccharide (LPS), which triggers immune response, induces a time-dependent increase of BDNF and microglia proliferation that was found to be dependent on A2ARs, which upon LPS increased the density [35]. Moreover, the enhanced secretion and proliferation of BDNF were reduced upon A2AR inhibition as well as after the removal of endogenous extracellular adenosine [35]. Brain samples of patients with Alzheimer's disease (AD) in microglia revealed the plaques surrounded by A2ARs, which were accompanied by upregulated cannabinoid CB2 receptors (CB2R). High levels of CB2Rs were also found in cultures of microglia that were exposed to LPS with IFN- γ [31]. The findings of Franco et al. (2019) suggest that A2ARs may be targets linked to the prevention of developing neurodegenerative disease, and the neuroprotective effect of their blockage is associated with enhanced cannabinoid signaling via CB2Rs [31].

The role of caffeine in neurodegenerative diseases

For a long period, findings of many epidemiological studies have been implying that coffee consumption lowers the risk of developing PD [49]. Although caffeine acts as a nonselective A1/A2 adenosine antagonist, the suggested neuroprotective effect is thought to be exerted by the blockage of A2ARs. Ishibashi et al. (2022) revealed that drinking one cup of coffee, equivalent to 100 mg of caffeine, leads to the substantial occupancy of A2ARs in the striatum, which is comparable with the one obtained by administration of 20–40 mg of istradefylline [49]. Istradefylline (KW-6002) is the first approved medication that selectively antagonizes A2ARs. In the US, it is currently indicated as an adjunctive treatment to levodopa-based regimens for adults with PD experiencing “off” time. The shared mechanism via A2AR inhibition is believed to underlie the beneficial effects of caffeine observed in PD.

The neuroprotective effect of A2AR antagonism in Parkinson’s disease

Istradefylline, the new A2AR antagonist medication was able to reduce the A53T- α -Syn-induced inclusion bodies, astrocyte activation, apoptosis, and the suppression of autophagic flux [44]. Hu et al., (2023) claim the blockage of A2AR promotes autophagic flux, degrades A53T- α -Syn protofibrils, and reduces neuronal toxicity and apoptosis [44]. Caffeine has also been reported to suppress the α -syn protein aggregates [135]. Luan et al., (2018) reported that chronic caffeine treatment suppresses a cascade of pathological events leading to α -synucleinopathy, including pSer129 α -Syn-rich aggregates, apoptotic neuronal cell death, microglia, and astroglia reactivation [70]. Xu et al., (2016) revealed that mice pretreated with caffeine presented with reduced losses of striatal dopamine and its transporters after the treatment with neurotoxic MPTP, which destroys dopaminergic neurons of SN. These effects of decreased MPTP-induced neurotoxicity were in line with the ones obtained by selective A2AR antagonists and ones observed in A2AR knockout mice [129]. According to Pierri et al., (2005) the neuroprotective effect of KW-6002 against MPTP neurotoxicity is related to the suppression of microglial activity in the substantia nigra, suggesting that antagonism of A2ARs reduces microglia activity [19,94]. Wilkaniec et al., (2017) observed that the genetic deletion of A2AR prevents the changes induced by α -syn, such as astrogliosis, NF- κ B activation, apoptotic neuronal cell death, abnormal synaptic plasticity, and memory deficits [126]. Consistently, Hu et al., (2016) have observed that A2AR knockdown inhibited the decline in dopamine levels, while blockage of these receptors prevented the lesions induced by α -syn [43]. Moreover, Giunta, Andriolo, and Castorina, (2014) reported that A2AR antagonist SCH58261 successfully prevented toxicity associated with β -amyloid proteins, the histopathological hallmarks of Alzheimer’s disease [33].

In the study of Wang et al., (2021) caffeine, by inducing autophagy in microglia, reduced the activation of NLRP3 inflammasome, which is the same inflammasome that becomes activated by α -synuclein aggregations during PD [125]. NLRP3 cleaves caspase-1, inducing the maturation of IL-1 β , which is later released from the cells [123]. Results of recent studies exhibited that NLRP3 activation can be suppressed by inducing transcription factor Nrf2 (Nuclear-factor-E2-related factor 2), which displays anti-oxidative properties [45]. Badshah et al., (2019) observed that the LPS-treated mice models of neurodegeneration presented with suppressed brain expression of Nrf2 and the enzyme heme oxygenase 1 (HO-1), whereas both Nrf2 and HO-1 were markedly upregulated in mice that were additionally administered caffeine [5]. By translocating to the nucleus, Nrf2 induces the expression of cytoprotective genes which are mainly involved in detoxification, antioxidation, and metabolism [123]. Nrf2 regulates the potential of the mitochondrial membrane and the availability of substrates required for respiration and ATP synthesis [123]. Moreover, without the presence of Nrf2, the

levels of NADPH are reduced [123]. Huang et al., (2024) revealed that induction of Nrf2 signaling by baicalin ameliorated MPTP and α -syn/MPP⁺ induced microglia activation, inflammatory response, and oxidative stress [45]. Furthermore, it minimized dopaminergic neuron loss and motor dysfunction [45]. Caffeine, by inducing signaling via anti-oxidative pathways, was able to upregulate the signaling of both nuclear factor erythroid 2-related factor 2 (Nrf2)-Keap1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), which further enhance the expression of transcription factors required to exert antioxidant and anti-inflammatory effects, and to control the function of mitochondria [106,102,135]. In A53T- α -syn neurons of the hippocampus, activated Nrf2 was able to improve the function of mitochondria and ameliorate the synaptic impairments [9]. In mice, genetic depletion of PGC-1 α suppressed the expression of genes responsible for mitochondrial respiration and reduced the activity of and decreased ATP levels [134]. In PGC-1 α knockout mice, treatment with MPTP neurotoxin resulted in a 61% loss of dopaminergic (TH-positive) neurons in SN, however in only a 12% loss in wild-type mice [134]. Moreover, administration of PGC-1 α -encoding adenovirus restored the induced by A53T- α -synuclein loss of DA neurons [134]. These mice presented with behavioral abnormalities displayed striatal neurodegenerative lesions that were associated with altered levels of ROS. PGC-1 α was reported to suppress ROS levels thus it is suggested that it may protect neurons under oxidative stress that is exerted during PD [110].

Zhang et al. (2021) demonstrated that the accumulation of intracellular ROS, followed by NLRP3 inflammasome activation in macrophages, correlates with the activity of the P2X7 receptor [132]. During neuroinflammatory conditions in CNS, P2X7R expression in microglia is enhanced, and extracellular ATP, evoked from damaged brain cells induces its activation [132]. P2X7R promotes the proliferation and activation of microglia, and directly induces neurodegeneration, via mediated by microglia neuronal death, glutamate-mediated excitotoxicity, and NLRP3 activation [132]. Dias et al., (2021) observed the P2X7R antagonist brilliant blue G (BBG) suppressed the upregulation of A2ARs, whereas caffeine by the blockage of A2AR downregulated the expression of P2X7R in the hippocampus and prefrontal cortex [22]. Moreover, both antagonists reduced the proinflammatory cytokines and markers of microglia activation [22].

The findings of Kang et al., (2012) imply that caffeine not only reduces the production of proinflammatory mediators, such as NO, PGE₂, and TNF- α but also suppresses their regulatory genes in LPS-stimulated BV2 microglial [53]. They suggest that caffeine inhibits the translocation of NF- κ B subunits, reducing the LPS-induced DNA-protein complex activity of NF- κ B, and attenuates phosphorylation of ERK, which they suppose underlines the main effector in gene regulation [53]. The expression levels of toll-like Receptor 4 (TLR4), phospho-nuclear factor kappa B (p-NF- κ B), and phospho-c-Jun n-terminal kinase (p-JNK) in the brains of mice injected with LPS were increased, however, their expression was downregulated in mice receiving LPS and caffeine [5]. Consistently, the genetic deletion of A2ARs attenuated neurodegeneration and cognitive impairments, reducing microglial and NF- κ B activation in the α -Syn fibril model of PD [43,127]. Moreover, LPS is known to induce the phosphorylation of ERK, JNK, and p38, however, pretreatment with caffeine only downregulated the LPS-induced ERK phosphorylation [53]. Caffeine has been also reported to enhance the proliferative PI3K/Akt signaling pathway [135]. Mohamed et al., (2016) observed that A2AR antagonist SCH58261 suppressed both intrinsic and extrinsic apoptotic pathways and caused the increase of Nrf-2, which is thought to be the effect of inhibited pERK1/2 signaling upon A2AR blockage [81]. Mohamed et al., (2016) observed that

intrahippocampal administration of A2AR antagonist SCH58261 reduced microglia activity in the hippocampus, suppressed the expression of glial TNF- α and BDNF, decreased glutamate, inducible nitric oxide synthase (iNOS) and thiobarbituric acid reactive substances (TBARS) [81]. Quiroz et al., (2006) demonstrated that inhibition of A2ARs with caffeine or a selective antagonist counteracts the signaling via cAMP–PKA and MAPK-ERK1/2 induced in response to corticostriatal afferents stimulation in vivo [98]. It is suggested that strong cortico-limbic-thalamic inputs to iSPNs allow the A2ARs to override the inhibitory effects of the D2R with significant activation of the cAMP–PKA cascade and MAPK [98]. In addition, LPS-induced NF- κ B activation is directly regulated through the phosphorylation of Akt, which is the main downstream molecule of phosphatidylinositol-3 kinase (PI3K), which has a significant role in cellular growth, adhesion, differentiation, and the inflammatory response [39]. Moreover, high levels of caffeine are able to stimulate apoptosis by increasing autophagy in cells of PC12D tumor cell lines via the PI3K/Akt/mTOR/p70S6 pathway [102].

Boia et al., (2017) demonstrated that A2AR blockage can prevent the pro-inflammatory signals that are induced in response to transient ischemia as observed in the retina [7]. Moreover, the effects exerted by caffeine were parallel with ones obtained by istradefylline [7]. They showed that 7-day reperfusion of caffeine significantly reduced the number of apoptotic cells and the levels of IL-1 β and TNF, even though it first caused their elevation [7]. Recent studies also found that caffeine has a striking ability to control the integrity and functionality of the blood-brain barrier, which is likely to be an effect operated by the abundant endothelial A2AR [20]. Pharmacological blockage of A2AR reduced the signaling via pERK1/2 and decreased glutamatergic transmission in the hippocampus of rats displaying ischemia [106]. As a result, it suppressed the inflammatory response, reducing TNF- α , NK- κ B, prostaglandin E2, and IL-6, with an increase in anti-inflammatory IL-10 [106].

The effect of A2AR antagonism on motor symptoms of Parkinson's disease

The neuroprotective effect of caffeine is closely associated with the observed improvement in motor function. This is believed to be induced by the blockage of A2ARs, as in many studies the results obtained by caffeine were comparable to ones of selective antagonists of A2ARs. In PD these receptors show upregulation and were associated with an increased risk of developing levodopa-induced dyskinesia [141,147]. Interestingly, it has been observed that the treatment with NMDAR antagonist, which prevented LID in MPTP-treated non-human primates, also lowered the level of A2ARs, suggesting their involvement in motor complications of dopaminergic treatment [141]. Moreover, in the study by Xiao et al., 6-OHDA-treated mice models of LID, exhibited a significant decrease in abnormal involuntary movements (AIMs) induced by L-DOPA upon conditional knockout of A2ARs in the forebrain [141,144]. Both caffeine and A2AR antagonist SCH 58261 significantly improved motor symptoms of rats after being treated with 6-OHDA, responsible for the induction of bradykinesia and motor disturbance [103]. Bibbiani et al. have reported that KW-6002, currently known as istradefylline, when administered together with dopamine receptor agonist, prevented the appearance of dyskinesia in MPTP-lesioned monkeys, which normally occurs upon 2 weeks of the treatment [141,145]. KW-6002 reduced the enhanced levels of GABA in GP of rats, after lesioning of the nigrostriatal pathway [142]. The blockage of A2AR suppressed the increased expression of enkephalin by iSPNs in 6-OHDA-treated animal models of PD [141,143]. Salamone, (2008) revealed that A2AR antagonists KW6002 and MSX-3 reduced the tremulous jaw movements, catalepsy, and enhanced locomotion in rat models of Parkinsonian tremor induced by D2 antagonist pimozide [146]. In addition, MSX-3 reduced the jaw movements caused by haloperidol and reserpine [146]. PET findings have

revealed that KW6002 reduced the loss of dopaminergic cells in rat models of PD and caused improvement in their performance [95]. Other animal models of PD, rotenone-induced rats, presented with a decreased activity of acetylcholinesterase and Na⁺/K⁺-ATPase, whereas elevated levels of TNF- α in the midbrain and the striatum. Models that were administered caffeine were more resistant to the effects of rotenone and did not display downregulation of the midbrain and striatal Na⁺/K⁺-ATPase, and caffeine significantly increased the activity of striatal acetylcholinesterase [55]. Moreover, caffeine was reported to stimulate the opening of K⁺ channels, preventing depolarization and neurotransmitter release, thus it is believed that caffeine may reduce the glutamatergic transmission onto iSPNs, reducing excitotoxicity.

In PD, the diminished signaling via D2Rs and the upregulated A2ARs result in the hyperactivity of iSPNs, followed by abnormal transmission via STN-GPi connection. This causes the 'NoGo' signals to predominate over the activity of the direct 'Go' pathway leading to the reduction in movement and development of Parkinsonian symptoms such as bradykinesia. Moreover, lack of dopaminergic inputs prevents the suppression of cholinergic interneurons, which in PD present with a hyperactive state. Antagonism of A2ARs, combined with levodopa treatment, is believed to restore the signaling via D2 receptors by reducing the tonic adenosinergic tone in both CINs and iSPNs, restoring the proper firing of STN. Domenici et al. (2004) for the first time demonstrated that antagonism of A2ARs suppresses the effects of mGlu5Rs stimulation in the rat striatum. In their studies, the A2AR antagonist ZM241385 fully prevented the effects of NMDA action potentiation induced by selective mGlu5R agonist (CHPG) [25,73].

Istradefylline as the first approved A2AR antagonist initiates a new approach to the treatment of Parkinson's disease by not only prolonging the action of levodopa but also interfering with the other altered pathway. The medication is administered together with dopamine precursor – levodopa. Contrary to expectations, monotherapy its efficiency in suppressing motor impairments in Parkinsonian patients was not significant as observed in one Phase II study, thus it was not further examined [28,50]. Although it was not effectively administered alone, once combined with even a low dose of L-DOPA, it may further suppress motor symptoms without worsening motor complications, preventing the need to increase the dopamine precursor dose over time [38]. Istradefylline when administered for 12 weeks as an adjunctive treatment to levodopa significantly reduced the change in daily OFF time compared with the placebo group in the study of 373 subjects (20 mg/day -0.99 hours, 40 mg/day -0.96 hours, placebo -0.23 hours) [58]. The same study was conducted for 52 weeks. The mean change in the daily OFF time from day 1 was - 0.65 hours in week 2, fluctuating between -0.71 and - 0.04 hours until week 52 in patients who had previously taken a placebo in the preceding double-blind study [58,80]. The most frequently reported treatment-emergent adverse events were nasopharyngitis and dyskinesia present in 24.4% and 21.4% of subjects respectively [58,80]. A study by Takahashi et al., (2024) has demonstrated that istradefylline treatment improved postural abnormalities (PAs) in Parkinsonian patients which was reflected by the decrease in the mean Unified Dystonia Rating Scale (UDRS) total score from 15.2 at baseline (week 0) to 10.2 at week 24 of the treatment [113]. The medication significantly improved the UDRS total score both in patients with and without therapy with dopamine agonists [113]. Furthermore, greater changes in the UDRS total score were observed in patients experiencing more severe PAs at baseline [113]. In the study consisting of 2719 patients, istradefylline was able to improve motor disability in both tremor dominant (TD) and postural instability and gait difficulty (PIGD) subtypes of Parkinson's disease [130]. Hatano et al., (2024) in the multicenter, open-label, randomized, parallel-group controlled study (ISTRA ADJUST PD)

demonstrated that istradefylline was able to reduce levodopa dose escalation in PD patients, resulting in lower cumulative levodopa use [37]. Therefore, the complications associated with increasing dopamine levels such as dyskinesias may be prevented from improving the quality of life [37]. It was supported by another study of 4026 patients by Hattori et al. As reported the slower levodopa dose escalation was present in patients who were initially treated with istradefylline, especially when combined with ≥ 600 mg/day of L-DOPA, supporting that A2AR antagonists may slow the progressive LDD increases [38]. Recent studies conducted by Waggan et al. (2022) revealed decreased availability of A2A receptor in the bilateral caudate nucleus in Parkinsonian patients with early stage of the disease in comparison with healthy controls ($P = 0.02$) [48]. Moderate-stage PD patients, on the other hand, showed its increased availability in the pallidum compared to healthy controls ($P=0.03$). Moreover, increased mean of striatal A2AR availability correlated with greater severity of motor symptom severity ($P = 0.02$) [48]. KW-6356, a novel A2AR antagonist and inverse agonist when administered to MPTP-treated marmosets significantly reversed motor impairments [87]. Its effectiveness in suppressing PD symptoms exceeded istradefylline and did not induce such dyskinesia. KW-6356 also enhanced the anti-parkinsonian effects of different doses of L-DOPA [88]. Studies on A2AR antagonists not only have revealed a reduction in striatal glutamatergic transmission when combined with “exogenous” D2R agonists but also after increasing the level of “endogenous dopamine”. Tozzi et al. (2012) revealed that the administration of cocaine, acting via DA transporter inhibition elevates intrastriatal DA levels [120]. Moreover, they demonstrated that co-administration of cocaine in ineffective doses together with a selective A2AR antagonist significantly enhances motor response in mice [120].

Kachroo (2005) revealed that the effects of acute administration of mGlu5R antagonist MPEP, combined with A2AR antagonist KW-6002 on locomotor activity are potentiated to a higher level than the sum of the effects of each drug alone. Moreover, the effect induced by MPEP was abolished in mGlu5R knockout mice, however amplified by an A2AR antagonist KW-6002, both in DA-depleted reserpinized mice and controls. The MPEP-induced response was suppressed in mice with single (A2A or D2) and double (A2A and D2) KO genotypes, whereas the motor stimulation by a D1 agonist was not attenuated in the KO mice, implying the importance of A2AR modulation in the treatment of PD [51].

Conclusion

At this moment there is no cure for Parkinson’s disease. Current pharmacotherapy aims to manage the motor impairments experienced by the patients, improving their quality of life and daily functioning. Levodopa replacement therapy is known to be the ‘gold standard’ in symptomatic parkinsonism, however, it has limitations. Approximately 80% of the treated PD patients develop levodopa-induced dyskinesia in the advanced stages of the disease. Istradefylline and other substances inhibiting adenosine 2A receptors, such as caffeine, act on A2ARs localized on both striatal and pallidal sides of the iSPNs and reduce their excitatory state induced by adenosine. Antagonism of A2ARs enhances the effects of dopaminergic stimulation on D2Rs, decreasing the signaling via hyperactive striatal indirect pathway, which leads to the improvement of motor function. The symptoms of PD are the representation of altered striatal activity that results from dopamine depletion associated with α -synuclein-mediated neuroinflammation. The findings of recent studies suggest that antagonism of A2ARs can alleviate the symptoms of PD and restore the physiological function of basal ganglia when combined with dopaminergic treatment. Moreover, the blockage of these receptors is thought to suppress the neuroinflammatory process and reduce the excitotoxicity.

In conclusion, the approval of istradefylline as an add-on treatment for patients with Parkinson's disease represents a significant advancement in the application of adenosinergic system modulatory medications, that may initiate a new approach to treating various medical conditions.

Disclosure

Author's contribution

Anna Wójcik – idea originator, graphics, conceptualization, writing-review, final edition, and supervision

Aleksander Górny – conceptualization, resources, writing-review

Justyna Chwiejczak – resources, graphics supervision and edition, verification of resources and data

Julita Młynarska- resources, verification of resources and data, language analysis, supervision

Jan Kościan- resources, data collection and analysis, writing manuscript

Karolina Szczerkowska- resources, supervision, visualization

Anna Seroka - resources, writing – original draft, writing manuscript

Maria Mitkowska- resources, writing – original draft

Maria Rybicka- resources, writing – original draft, writing manuscript

Michał Obrębski- resources, supervision, writing – original draft

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