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Pharmacological interactions between adenosine A_{2A} receptor antagonists and different neurotransmitter systems

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ABSTRACT

While Parkinson's disease (PD) is traditionally characterized by dopaminergic neuron degeneration, several neurotransmitters and neuromodulators besides dopamine are also involved in the onset and progression of the disease and its symptoms. The other principal neurotransmitters/neuromodulators known to control basal ganglia functions and, in particular, motor functions, are GABA, glutamate, serotonin (5-HT), noradrenaline, acetylcholine, adenosine and endocannabinoids. Among these, adenosine is the most relevant, acting through its adenosine A_{2A} receptor. Work in experimental models of PD has established the effects of A_{2A} receptor antagonists, including the alleviation of disrupted dopamine functions and improved efficacy of dopamine replacement therapy. Moreover, positive interactions between A_{2A} receptor antagonists and both D_2 and D_1 receptor agonists have been described *in vitro* at the receptor–receptor level or in more complex *in vivo* models of PD, respectively. In addition, the interactions between A_{2A} receptor antagonists and glutamate ionotropic $GluN_{2B}$-containing *N*-Methyl-D-aspartic acid receptors, or metabotropic glutamate (mGlu) receptors, including both $mGlu_5$ receptor inhibitors and $mGlu_4$ receptor activators, have been reported in both *in vitro* and *in vivo* animal models of PD, as have positive interactions between A_{2A} and endocannabinoid CB_1 receptor antagonists. At the same time, a combination of A_{2A} receptor antagonists and 5-HT_{1A}–5-HT_{1B} receptor agonists have been described to modulate the expression of dyskinesia induced by chronic dopamine replacement therapy.

1. Introduction

Adenosine is extensively distributed in the central nervous system, where it acts through several metabotropic adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, all of which have different distributions and levels of expression [1]. Of these, A_{2A} receptors are abundant in the basal ganglia and are specifically concentrated in the GABAergic striatopallidal indirect output pathway, which controls, smooths and coordinates movement to exert an inhibitory effect on the thalamocortical system [2]. Due to the positioning of A_{2A} receptors, and their interaction with dopamine receptors, the efficiency of A_{2A} receptor antagonists in counteracting the cardinal symptoms of Parkinson's disease (PD) has been tested [3]. Preclinical studies and clinical trials have consistently shown that A_{2A} receptor antagonists are effective in reducing the '*wearing-off*' phenomenon, and increasing the duration of '*ON*' periods [4–6]. However, differently from preclinical studies [4,5], multicenter clinical trials [7–10] have reported an increased incidence of levodopa-induced dyskinesias (LID), classified as non-troublesome, during the treatment with the A_{2A} receptor antagonist istradefylline [7–10]. To date, istradefylline is marketed in Japan and the USA as an adjunct to levodopa to attenuate *wearing-off* in advanced PD patients [11,12]. Nowadays, A_{2A} receptor antagonists are considered a very promising therapy for PD.

1.1. Interactions between dopamine and A_{2A} receptor antagonists

Preclinical studies have shown that the co-expression of adenosine A_{2A} and dopamine D_2 receptors in striatopallidal neurons provides the anatomical basis for their functional antagonistic interactions [13] and thus the antiparkinsonian effects of A_{2A} receptor antagonists (Fig. 1). Similar to dopamine D_2 receptor stimulation, adenosine A_{2A} receptor blockade decreases the excessive inhibitory output of the striatal indirect pathway [13]. In addition, a critical role of A_{2A} receptors has been

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demonstrated in the medium spiny neurons terminating in the globus pallidus (GP), where antagonism of pallidal A_{2A} receptors has been shown to decrease extracellular GABA release thereby contributing to the reinstatement of GP and, in turn, subthalamic (STN) activity [14]. On the basis of these studies [13–15], interactions between adenosine A_{2A} and dopamine receptors seem to be responsible for most of the motor activation produced by A_{2A} receptor antagonists. Moreover, the synergistic actions of A_{2A} antagonists and dopamine agonists is very evident in animal models of PD where a marked efficacy in relieving cardinal PD motor symptoms is observed [3,16]. However, the D_1-receptor-containing pathway directly projecting to the substantia nigra pars reticulata is also highly involved in the regulation of the motor behavior. Therefore, by acting at the network level, blockade or stimulation of the adenosine A_{2A} receptors in the striatopallidal pathway may also indirectly facilitate or worsen dopaminergic D_1-mediated responses, respectively [13,17].

In addition, close physical interactions between dopamine D_2 and A_{2A} receptors has been demonstrated through co-immunoprecipitation and co-localization assays, as well as fluorescence resonance energy transfer 'FRET' and bioluminescence resonance energy transfer 'BRET' techniques in co-transfected cells [18,19] (Fig. 1). This interaction between D_2 and A_{2A} receptors has been suggested by some authors to play an important role in the efficacy of A_{2A} receptor antagonists in PD [18,19]. Others suggest that the main actions of A_{2A} receptors are mediated through a dual excitatory modulation of the GABAergic indirect pathway (see review by Mori in this supplement). In this review, we describe how interactions with several other neurotransmitters/neuromodulators also affect the potential efficacy of A_{2A} receptor antagonists against both PD symptoms and PD-associated dyskinesia produced by long-term levodopa therapy [20,21].

1.2. Interactions between glutamate and A_{2A} receptor antagonists

In the basal ganglia, the glutamatergic and dopaminergic systems play an essential role in the modulation of motor and goal-directed behaviors originating in the caudate-putamen (CPu) [2,22]. In PD, the loss of dopamine promotes the hyperactivation of the indirect striatal pathway and, in particular, of the glutamatergic STN, two events, that contribute to the development of PD motor disabilities [23].

From this evidence, numerous studies have been performed to assess the antiparkinsonian activity of compounds that selectively inhibit glutamatergic neurotransmission [24]. Despite the fact that several antagonists for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-Methyl-D-aspartic acid (NMDA) ionotropic glutamate (iGlu) receptors have demonstrated antiakinetic and antidyskinetic properties (for review see [25,26]), AMPA and NMDA receptors are widely expressed in the central nervous system, raising concern regarding potential adverse psychiatric and cognitive effects [27].

Interestingly, NMDA receptors carrying the $GluN_{2B}$ subunit have a relatively restricted distribution and are enriched in the basal ganglia

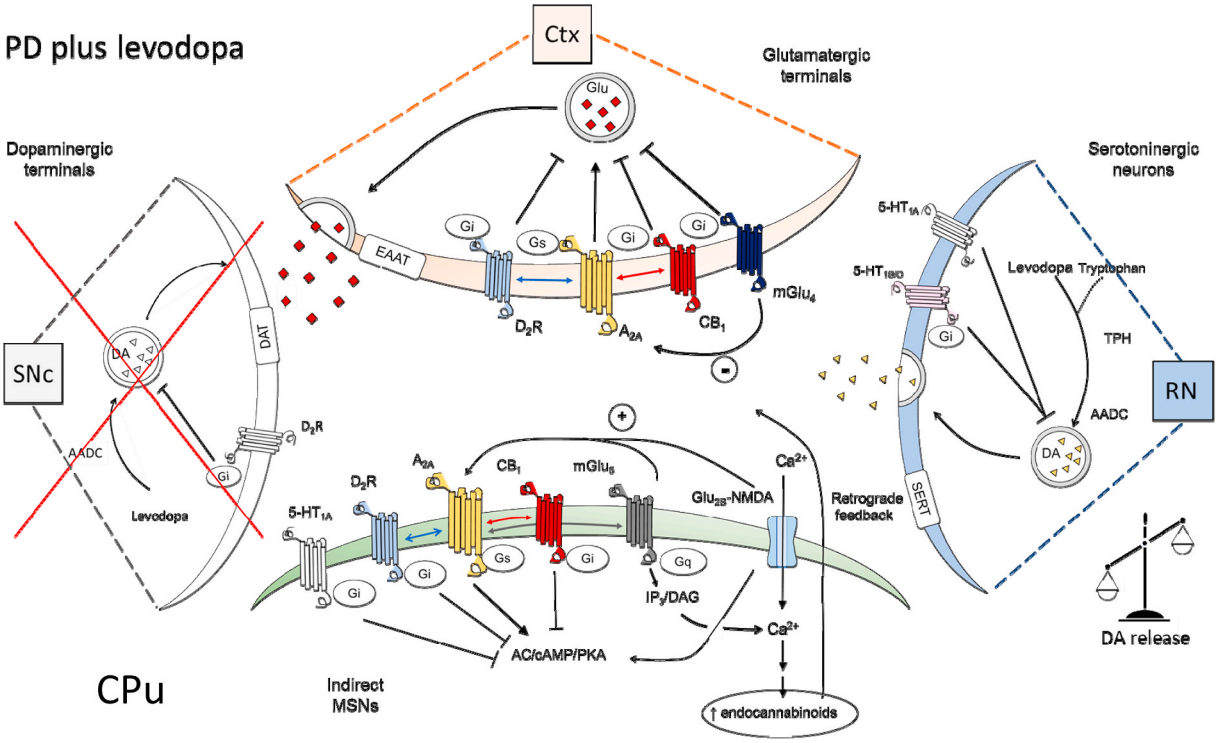

Fig. 1. Schematic representation of A_{2A} receptor functions and interactions with glutamatergic, dopaminergic, serotoninergic and endocannabinoid receptors in the caudate-putamen.

PD in the basal condition. In PD, the decrease of dopaminergic terminals results in the increase of glutamate release from cortical terminals. At the presynaptic level, A_{2A} receptors negatively interact with the $mGlu_4$ receptor in the intracellular signaling cascade, as well as with D_2 and CB_1 receptors, by establishing receptor-receptor interactions. At the postsynaptic level, the stimulation of A_{2A} receptors on iMSNs increases the firing probability of GABAergic striatopallidal neurons, counteracting D_2-and CB_1-mediated inhibition. In addition, A_{2A} receptors positively interact with glutamatergic $mGlu_5$ and Glu_{2B}-containg NMDA receptors, and establish direct receptor-receptor interactions with CB_1, D_2, and $mGlu_5$ receptors. At the iMSN level, stimulation of $mGlu_5$ and Glu_{2B}-containing NMDA receptors triggers the generation of inhibitory retrograde feedback mediated by endocannabinoids.

Colored, double-headed arrows indicate the different A_{2A} receptor-receptor interactions that lead to the formation of heteroreceptors with CB_1 (red), D_2 (light blue), and $mGlu_5$ (grey) receptors. Abbreviations: AADC, amino acid decarboxylase; AC, adenylyl cyclase; cAMP, 3′,5′-cyclic adenosine monophosphate; CB_1, endocannabinoid 1; CPu, caudate-putamen; Ctx, cortex; DA, dopamine; DAG, diacylglycerol; DAT, dopamine transporter; EAAT, excitatory amino acid transporter; Glu, glutamate; IP_3, inositol trisphosphate; iMSNs, indirect medium spiny neurons; NAM, negative allosteric modulator; PAM, positive allosteric modulator; PKA, phosphokinase A; RN, raphe nucleus; SERT, serotonin transporter; SNc, substantia nigra pars compacta; TPH, tryptophan hydroxylase.

regions [28] (Fig. 1), supporting the notion that antagonists selective for $GluN_{2B}$ NMDA receptors may possess less unwanted effects and produce higher therapeutic actions in brain regions relevant to PD pathophysiology. On this topic, preclinical studies revealed that the administration of $GluN_{2B}$ NMDA receptor antagonists significantly ameliorated parkinsonian-like motor deficits observed in both unilateral 6-hydroxydopamine (6-OHDA)-lesioned (hemiparkinsonian) rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys, especially when coadministrated with levodopa [29–31]. In addition, blockade of $GluN_{2B}$ NMDA receptors was found to contrast LID in MPTP-treated monkeys [32,33]. It is important to mention that contrasting preclinical and clinical results have also been reported which questioned the therapeutic effects of $GluN_2B$ NMDA receptor antagonists in PD treatment [34–38]. Nonetheless, recent encouraging findings obtained in rodent and non-human primate models of PD revealed that the combined blockade of $GluN_2B$ NMDA and A_{2A} receptors may significantly improve motor performances without producing any of the motor complications associated with levodopa [39,40], opening new opportunities for future clinical studies (Fig. 2). In this respect, it has been proposed that A_{2A} and NMDA receptors located in the CPu may share a common intracellular signaling pathway, given that higher levels of cAMP were observed following the stimulation of either striatal A_{2A} or NMDA receptors, especially when nigrostriatal terminals were removed [41] (Fig. 1). Besides, a negative interaction between A_{2A} and NMDA receptors at the electrophysiological level has also been reported, which could be involved in the above-mentioned pharmacological effects [42,43].

Apart from iGlu receptors, metabotropic glutamate (mGlu) receptors have also been extensively explored as a potential target for the treatment of PD and LID (for review see [44,45]). mGlu receptors are highly expressed in basal ganglia networks, where they are involved in the regulation of synaptic transmission and plasticity [46]. mGlu receptors include eight subtypes that have been divided into three groups (groups I, II, and III), based on their sequence homologies, specific coupling with G proteins, and ligand-binding profiles [47]. Group I mGlu ($mGlu_1$ and $mGlu_5$) receptors are principally postsynaptic, and their activation increases neuronal excitability; whereas, group II ($mGlu_2$ and $mGlu_3$) and III mGlu ($mGlu_4$, $mGlu_7$, $mGlu_8$) receptors are located presynaptically, where their stimulation decreases the probability of neurotransmitter release [48]. Among mGlu receptors, the $mGlu_5$ and $mGlu_4$ receptors have consistently proven to be the most promising pharmacological target in PD therapy. Studies performed in bilaterally 6-OHDA-lesioned rats revealed as MPEP, a non-competitive $mGlu_5$ receptor antagonist, significantly alleviated bradykinesia when injected chronically [49–51]. In addition, administration of either MPEP or MTEP, $mGlu_5$ negative allosteric modulators (NAMs), was found to significantly reduce LID both in hemiparkinsonin rats [52] and MPTP-treated monkeys [53–55], and to attenuate the neurodegeneration of nigrostriatal neurons [56,57]. Apart from $mGlu_5$ receptors, agonists and positive allosteric modulators (PAMs) of $mGlu_4$ receptors demonstrated encouraging antiparkinsonian

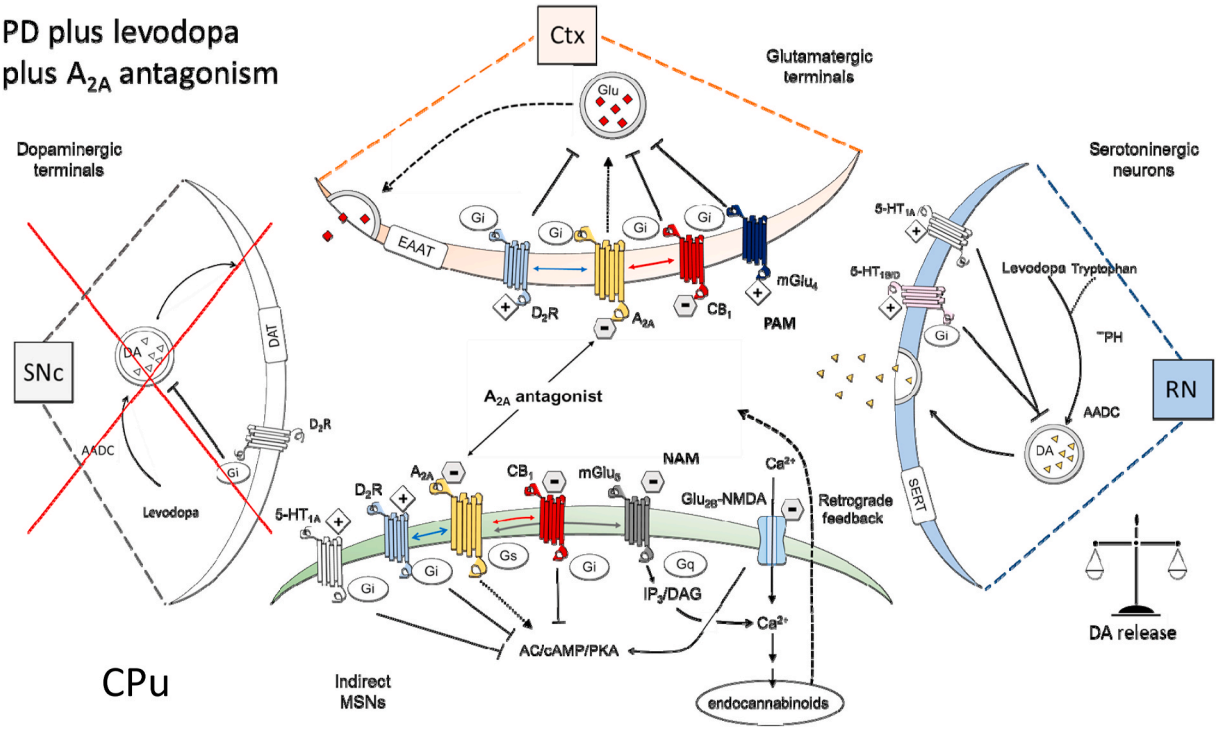

Fig. 2. Schematic representation of A_{2A} receptor functions and interactions with glutamatergic, dopaminergic, serotoninergic and endocannabinoid receptors in the caudate-putamen.

PD after A_{2A} receptor antagonism. Levodopa is transformed into dopamine in 5-HT neurons, which partially restores the striatal indirect pathway outflow through D_2 receptor stimulation. However, due to the lack of physiological release from 5-HTergic terminals, levodopa administration eventually leads to the development of dyskinesia. The combined stimulation of $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ receptors, located in iMSN dendrites and 5-HTergic terminals, reduces dyskinesia by lowering iMSN activity and stabilizing dopamine release. Blockade of A_{2A} receptors reduces the excessive release of glutamate from cortical terminals and lessens the excitation of iMSNs, leading to an improvement in motor performance. These antiparkinsonian effects are amplified by PAM-mediated activation of $mGlu_4$ receptors, and NAM-mediated inhibition of $mGlu_5$ receptors, as well as the antagonism of Glu_{2B}-containing NMDA receptors on iMSNs, which further lower the excitation of cortical and striatopallidal neurons. Colored, double-headed arrows indicate the different A_{2A} receptor-receptor interactions that lead to the formation of heteroreceptors with CB_1 (red), D_2 (light blue), and $mGlu_5$ (grey) receptors; grey hexagons represent receptor antagonists and NAMs; white rhombuses represent PAMs. Abbreviations: 5-HT, serotonin; AADC, amino acid decarboxylase; AC, adenylyl cyclase; cAMP, 3′,5′-cyclic adenosine monophosphate; Ctx, cortex; CPu, caudate-putamen; DA, dopamine; DAG, diacylglycerol; DAT, dopamine transporter; EAAT, excitatory amino acid transporter; Glu, glutamate; IP_3, inositol trisphosphate; iMSNs, indirect medium spiny neurons; NAM, negative allosteric modulator; PAM, positive allosteric modulator; PKA, phosphokinase A; RN, raphe nucleus; SERT, serotonin transporter; SNc, substantia nigra pars compacta; TPH, tryptophan hydroxylase.

effects when administrated in combination with levodopa. Increased activation of $mGlu_4$ receptors was found to reverse akinesia in haloperidol-treated rats [58–60] and to potentiate levodopa motor effects in both hemiparkinsonian rats [60] and MPTP-treated monkeys [61]. Additionally, a significant reduction of LID was also reported [61].

Interestingly, a growing amount of evidence has suggested that administration of A_{2A} receptor antagonists act synergistically to potentiate the therapeutic effects of both $mGlu_5$ receptor inhibitors and $mGlu_4$ receptor activators (Fig. 2). In this respect, *in vitro* experiments performed in isolated striatal primary neurons have demonstrated that transcriptional cellular profiles were significantly modified by the simultaneous stimulation of D_2, A_{2A} and $mGlu_5$ receptors [62]. In addition, some experiments have found that the combined systemic administration of different A_{2A} antagonists with MPEP synergistically improved parkinsonian-like motor deficits in rats either carrying a unilateral or bilateral 6-OHDA lesion or in rats pre-treated with reserpine [49,63,64](Fig. 2).

Moreover, antiparkinsonian effects and synergistic interactions have been reported between A_{2A} receptor antagonists and $mGlu_4$ receptor activators both in hemiparkinsonian or haloperidol-treated rats [65,66] (Fig. 2). The counteraction of dopamine inhibition in striatopallidal neurons by the activation of postsynaptic A_{2A}-$mGlu_5$ heteroreceptors [67–69], combined with the tight regulation exerted by A_{2A} and $mGlu_4$ receptors during the release of GABA and glutamate from striatopallidal and corticostriatal terminals, respectively [65], strongly indicate the critical role of these receptors in the modulation of the striatal indirect pathway activity, although the exact mechanisms involved are still unknown. Altogether, this preclinical evidence emphasizes the therapeutic potential of this integrative, non-dopaminergic pharmacological approach in PD therapy, and provides support for further studies in preclinical and clinical settings.

1.3. Interactions between endocannabinoids and A_{2A} receptor antagonists

The selective localization and high expression of A_{2A} and endocannabinoid (CB_1) receptors in the CPu have inspired several studies on the potential interactions between these receptors, including their modulation of motor activity [70,71]. These two receptors are in fact co-localized in fibrillar structures of the CPu, which represent either dendritic processes or nerve terminals [72] (Fig. 1). In particular, the study by Ref. [72] has shown that signaling mediated by CB_1 receptors is completely dependent on the activation of A_{2A} receptors in the human neuroblastoma cell line and the motor-depressant effect produced by the striatal administration of CB_1 receptor agonists in rats is counteracted by systemic administration of A_{2A} receptor antagonists, providing an *in vivo* behavioral correlate for the above mentioned biochemical results [72]. These studies have therefore suggested that drugs antagonizing the endocannabinoid CB_1 receptor might be beneficial in PD [73,74].

Many studies have shown that progression of substantia nigra *pars compacta* neuron degeneration, as well as the appearance of major parkinsonian symptoms, seem to be associated with over-activity of the endocannabinoid signaling system (e.g., increased CB_1 receptor density and function and elevated endocannabinoid levels in most basal ganglia nuclei), both in animal models and in PD patients [74]. However, administration of the CB_1 receptor antagonist rimonabant had no effect on bradykinesia or posture in a non-human primate model of PD [75]. Therefore, blockade of CB_1 receptors might only be effective in particular circumstances of PD (e.g. in advanced phase of the disease), although further clinical studies are needed to elucidate these aspects [76].

Interestingly, the apparent requirement of A_{2A} receptor activation to mediate the motor-depressant effects of endocannabinoids appears to be dependent on the existence of A_{2A}-CB_1 heteromeric receptor complexes [72,77] (Fig. 1). It is possible that dimeric and/or multimeric receptor complex formation is involved in the mechanism by which A_{2A} and non-dopaminergic receptors interact in the CPu [69,77,78].

In addition, the inhibitory effect of CB_1 receptor agonists on hyperlocomotion induced by the D_2 agonist quinpirole was blocked by either the CB_1 receptor antagonist rimonabant or the A_{2A} receptor antagonist MSX-3. This provides evidence for the existence of antagonistic interactions within CB_1–D_2 receptor heteromers, in which A_{2A} receptors may also participate [79] (Fig. 1). These results were confirmed by the development of Sequential Resonance Energy Transfer 'SRET', which allowed the detection of heteromultimers [80]. Therefore, even though some reports support the idea that the main function of endocannabinoid signaling within the basal ganglia circuitry involves retrograde signaling in GABAergic and glutamatergic synapses (reviewed in Ref. [73]) (Fig. 1), the existence of postsynaptic CB_1-containing heteromers provides new indications regarding the potential mechanisms underlying the endocannabinoid-mediated control of striatal function, as these heteromers cannot just be considered as retrograde effectors that inhibit neurotransmitter release [77].

To further determine the therapeutic efficacy of multidrug treatment, in the hemiparkinsonian model of PD, Pinna and colleagues [81], administered levodopa with compounds that act upon A_{2A}–CB_1–D_2 heteromers, namely A_{2A} (MSX-3 or SCH5 8261) and CB_1 (rimonabant) receptor antagonists (Fig. 2). In this study, it was found that the combined administration of an A_{2A} receptor antagonist with rimonabant produced significant potentiation of contralateral turning compared with that induced by levodopa alone. This enhancement, however, was not significantly different from that induced by administration of levodopa plus an A_{2A} receptor antagonist. This suggests that the lack of additional antiparkinsonian efficacy of CB_1 antagonist might be due to levodopa treatment, which might disrupt the interactions among A_{2A}, CB_1, and D_2 receptors, thus eliminating the beneficial effects of such interactions. These results are in accordance with other studies in non-human primates, in which the expression of A_{2A}–CB_1, A_{2A}–D_2, and CB_1–D_2 heteromers was reduced in the CPu of monkeys that received chronic levodopa treatment, suggesting possible alteration of heteromer expression during the course of human disease [82].

However, it is noteworthy that A_{2A} receptor antagonists display not only different striatal pre- and postsynaptic profiles, but also a differential affinity for receptors depending on the partner (or partners) of the receptor heteromer [83,84]. Therefore, although radioligand binding data have shown A_{2A}–CB_1–D_2 receptor heteromers to be present in the CPu of both naïve and hemiparkinsonian rats, further studies using different A_{2A} antagonists are required to clarify the behavioral influence of combined administration of A_{2A} and CB_1 antagonists in physiological and neuropathological conditions [81].

1.4. Interactions between A_{2A} receptor antagonists and serotonin receptors

In the brain, serotonin (5-HT) neurons originate in the raphe nuclei and project to different brain areas, including the cerebral cortex, limbic structures, diencephalon, spinal cord and, most importantly, the basal ganglia [85]. The 5-HTergic system is associated with numerous functions, including emotion, cognition and motor behavior, suggesting that changes in 5-HTergic neurotransmission may contribute to PD-related motor and non-motor symptoms [86]. Besides contributing to motor and non-motor symptoms, the 5-HTergic innervation of the CPu represents the main site of dopamine production and release after levodopa administration in the advanced stages of PD [87,88] (Fig. 1). Thus, the 5-HTergic system contributes to the onset of LID by promoting the non-physiological release of dopamine [87,88]. In the early stages of PD, levodopa is converted into dopamine, then released and reuptaken steadily in the residual presynaptic dopaminergic terminals. In advanced stages of PD, where dopaminergic innervation is almost totally lost, levodopa is mostly converted into dopamine in non-dopaminergic neurons, such as 5-HTergic neurons. Indeed, 5-HTergic neurons contain the complete enzymatic set required for transformation of exogenous levodopa into dopamine [88,89] (Fig. 1). However, these

neurons possess neither dopamine reuptake receptors nor dopamine autoreceptors. Thus, after levodopa administration, the dopamine released from 5-HTergic terminals reaches an excessive concentration peak and, after pulsatile oral levodopa doses, extreme dopamine fluctuations occur. These events may cause the development of dyskinesia [88,90]. Supporting this finding, several studies have demonstrated that toxin-induced lesions or pharmacological silencing of 5-HT neurons are able to suppress LID in dyskinetic rat and macaque models [87,91].

Among the seven families of 5-HT receptors that mediate 5-HTergic transmission, the 5-HT_{1A} and 5-HT_{1B} subtypes are likely to play the main role in dyskinesia expression [88,92] (Fig. 1). These receptors are located on both presynaptic and postsynaptic neurons, including striatal spiny projection neurons, 5-HTergic neurons of the raphe nuclei and glutamatergic corticostriatal projections, and regulate the release of neurotransmitter from 5-HTergic neurons [92]. Specifically, the activation of presynaptic 5-$HT_{1A/1B}$ receptors reduces the occurrence of extreme dopamine release after levodopa administration, and thus attenuates dyskinesia [88,93]. Moreover, activation of postsynaptic 5-HT_{1A} receptors in the cerebral cortex and CPu seems to alleviate LID by inhibiting both the activity of corticostriatal glutamatergic neurons and the striatal medial spiny neurons [94]. In addition, the 5-HT_{1B} receptors expressed in medium spiny neurons are significantly upregulated by levodopa [95]. According to these findings, certain 5-HT_{1A} and 5-HT_{1B} receptor agonists, such as buspirone, anpirtoline, and eltoprazine, show antidyskinetic effects in rat and primate models of dyskinesia, as well as in PD patients [93,96–98]. However, these drugs have also been found to decrease the antiparkinsonian efficacy of levodopa in PD animal models [96,97]. Several ongoing clinical trials of 5-HT_{1A} and 5-$HT_{1A/1B}$ agonists such as eltoprazine, buspirone, and piclozotan are currently being undertaken in advanced PD patients by various pharmaceutical companies [93,98,99].

As shown in preclinical previous studies, A_{2A} receptor antagonists administered chronically along with a low dose of levodopa potentiate the motor efficacy of levodopa, and extend its duration of effect without contrasting or exacerbating dyskinesia, in comparison to a full dose of levodopa [4,16,77,78,100] (Fig. 2). On the basis of these findings, recent studies have attempted to develop a pharmacological therapy that could mitigate dyskinesia without worsening PD disability. These studies have explored the efficacy of combined treatment with the partial 5-$HT_{1A/1B}$ agonist, eltoprazine, and the adenosine A_{2A} receptor antagonist, preladenant, in mitigating dyskinesia and dyskinetic movements in both the hemiparkinsonian rat and MPTP-treated primate models of PD [101,102].

The results of these studies have provided a new treatment possibility involving A_{2A} receptor antagonists, since combined acute administration of eltoprazine plus preladenant was found to counteract LID without affecting contralateral rotational behavior, which appeared as a fluid, rather than stereotyped, movement. Moreover, evaluation of total motor activity confirmed that no motor impairment was displayed by hemiparkinsonian rats treated with levodopa in combination with eltoprazine plus preladenant [101]. Notably, this triple drug combination not only preserved rotational behavior, but also prevented the worsening of motor and sensorimotor performance, which was induced by eltoprazine plus levodopa in hemiparkinsonian rats during specific tests, including the adjusting step test, initiation time of stepping, and vibrissae-evoked forelimb-placing test [78,101]. Furthermore, chronic treatment using the combination of eltoprazine, preladenant, and levodopa in hemiparkinsonian rats produced a permanent reduction of LID and preserved rotational behavior in rats that were already dyskinetic [101]. Moreover, this triple drug combination prevented the onset of LID in levodopa-naïve hemiparkinsonian rats [101]. Therefore, this combination of drugs produced a near-complete and long-lasting suppression of LID without worsening motor responses, (unlike eltoprazine in the same behavioral tests) [78,97,101]. However, the effects of the eltoprazine, preladenant, and levodopa combination were slightly different in dyskinetic MPTP-treated primates. While a high dose of eltoprazine worsened their disability scores, a moderate dose of eltoprazine appeared to reduce their dyskinesia, without affecting the therapeutic efficacy of levodopa. Interestingly, combined administration with preladenant prevented the detrimental effect that the high dose of eltoprazine had on the disability and bradykinesia scores. However, it should be noted that preladenant was found to partially worsen the antidyskinetic effect of eltoprazine, at least when coadministered with a full dose of levodopa. By contrast, combined administration of preladenant and eltoprazine, along with a suboptimal dose of levodopa, counteracted dyskinesia and maintained the full therapeutic effects of levodopa. Ko and colleagues [102] confirmed that repeated treatment with the combination of preladenant, eltoprazine, and a subthreshold dose of levodopa, did not worsen motor disability in parkinsonian primates. These combined drug treatments seemed to increase time in the ON state and appeared to initially maintain lower levels of dyskinesia; however, these beneficial effects were not maintained by the end of the two weeks of treatment. This suggests that this drug combination could be very useful to contrast acute dyskinetic episodes [102].

The fact that a direct adenosine A_{2A}/5-HT_{1A} receptor interaction has been demonstrated in co-transfected cells [103] must be considered when evaluating mechanisms behind the effects elicited by the combination of preladenant and eltoprazine (Fig. 2). However, no evidence of A_{2A}/5-HT_{1A} receptor-receptor interaction has been shown so far *in vivo*. Other possible mechanisms may involve the pharmacological interaction between eltoprazine and preladenant. Indeed, it may be hypothesized that, while activation of presynaptic 5-$HT_{1A/1B}$ receptors may dampen the excessive dopamine release from 5-HT neurons, thus reducing dyskinesia [88]; A_{2A} receptor antagonists may potentiate motor activation through postsynaptic interaction between A_{2A} and dopamine receptors [5,6]. Overall, these findings propose a new, non-dopaminergic pharmacological strategy for the treatment of dyskinesia in PD, involving A_{2A} and 5-HT receptors.

2. Conclusions

Due to the complexity of PD and the several side effects that long-term dopamine replacement therapy produces, adenosine A_{2A} receptor antagonists could be an important add-on therapy to counteract the *wearing-off* phenomenon and increase ON time without any exacerbation of troublesome dyskinesia in complicated PD patients. These effects might be mediated by the well-known dopamine-A_{2A} receptor interaction, but several other interactions involving A_{2A} receptors and other neurotransmitters and neuromodulators described in this review may also be involved, including glutamate, 5-HT and endocannabinoid receptors, which all deserve greater attention in future preclinical and clinical studies.

Author contributions

Micaela Morelli, Annalisa Pinna designed the manuscript. Micaela Morelli, Annalisa Pinna, Marcello Serra, and Jacopo Marongiu wrote, revised and edited the manuscript. Marcello Serra realized the figures. All authors approved the final manuscript.

Disclosure statement

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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