Functional and Neuroprotective Role of Striatal Adenosine A_{2A} Receptor Heterotetramers

Sergi Ferré, MD, PhD¹ and Francisco Ciruela, PhD^{2,3}

In the striatum, adenosine A_{2A} receptors (A_{2A}R) are mainly expressed within the soma and dendrites of the striatopallidal neuron. A predominant proportion of these striatal postsynaptic A_{2A}R form part of the macromolecular complexes that include A_{2A}R-dopamine D₂ receptor (D₂R) heteromers, G_{olf} and G_{i/o} proteins, and the effector adenylyl cyclase (AC), subtype AC5. The A_{2A}R-D₂R heteromers have a tetrameric structure, constituted by A_{2A}R and D₂R homomers. By means of reciprocal antagonistic allosteric interactions and antagonistic interactions at the effector level between adenosine and dopamine, the A_{2A}R-D₂R heterotetramer-AC5 complex acts as an integrative molecular device, which determines a switch between the adenosine-facilitated activation and the dopamine-facilitated inhibition of the striatopallidal neuron. Striatal adenosine also plays an important pre-synaptic modulatory role, driving the function of corticostriatal terminals. This control is mediated by adenosine A₁ receptors (A₁R) and A_{2A}R, which establish intermolecular interactions forming A₁R-A_{2A}R heterotetramers. Here, we review the functional role of both presynaptic and postsynaptic striatal A_{2A}R heterotetramers as well as their possible neuroprotective role. We hypothesize that alterations in the homomer/heteromer stoichiometry (i.e., increase or decrease in the proportion of A_{2A}R forming homomers or heteromers) are pathogenetically involved in neurological disorders, specifically in Parkinson's disease and restless legs syndrome.

Keywords: adenosine A_{2A} receptor, adenosine A₁ receptor, dopamine D₂ receptor, GPCR heteromers, Parkinson's disease, restless legs syndrome

Introduction: Precoupling and Oligomerization

PRECOUPLING OF G protein-coupled receptors (GPCR) with G proteins and signaling molecules and receptor oligomerization are two concepts that are changing our classical views of GPCR physiology and pharmacology.^{1,2} The classical view of freely moving molecules of GPCR, G proteins, and effectors in the plasma membrane, which establish ligand-guided associations and dissociations by random collision (collision-coupling mode), is being replaced by a ligand-induced rearrangement of precoupled molecules.¹ Furthermore, the classical view of single GPCR units is being replaced by oligomeric functional units. A large number of experimental data support the view of a common functional building block constituted by two GPCR units (homodimer) and

one heterotrimeric G protein (with its preferred α and $\beta\gamma$ subunits).³

A GPCR heteromer is defined as “a macromolecular complex composed of at least two (functional) receptor units (protomers) with biochemical properties that are demonstrably different from those of its individual components.”⁴ It is becoming apparent that a common quaternary structure of GPCR heteromers is a heterotetramer, formed by two different GPCR homodimers coupled to their cognate G proteins.^{1,3} This has been recently reported as the most probable quaternary structure of two striatal adenosine A_{2A} receptor (A_{2A}R) heteromers, the postsynaptic A_{2A}R-dopamine D₂ receptor (D₂R) heterotetramer and the presynaptic adenosine A₁ receptor (A₁R)-A_{2A}R heterotetramer.^{4,5} Here, we review the functional role of both heterotetramers as well as their possible neuroprotective role.

¹Integrative Neurobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, Maryland.

²Pharmacology Unit, Department of Pathology and Experimental Therapeutics, School of Medicine, IDIBELL, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain.

³Institute of Neurosciences, University of Barcelona, Barcelona, Spain.

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Thus, we hypothesize that alterations in the homomer/heteromer stoichiometry (i.e., increase or decrease in the proportion of GPCR protomers forming homomers or heteromers) are pathogenetically involved in neurological disorders, specifically in Parkinson's disease (PD) and restless legs syndrome (RLS).

Functional Role of the Postsynaptic Striatal A_{2A}R-D₂R Heterotetramer

In the striatum, A_{2A}R and D₂R are mostly expressed within the soma and dendrites of the striatopallidal neuron, one of the two subtypes of GABAergic efferent neurons that constitute more than 95% of the striatal neuronal population.⁶ A predominant proportion of these striatal postsynaptic A_{2A}R and D₂R form part of the macromolecular complexes that include A_{2A}R-D₂R heterotetramers, G_s

(more properly G_{olf} subtype, but G_{s/olf} proteins are referred as G_s proteins, for short) and G_{i/o} proteins (G_i proteins, for short), and the effector adenylyl cyclase (AC) subtype AC5 (Fig. 1).^{5,7} These complexes provide the frame for the canonical G_s-G_i antagonistic interaction at the AC level (Fig. 1A). This canonical interaction implies the ability of an activated G_i-coupled receptor to inhibit a G_s-coupled receptor-mediated AC activation,⁸ thus requiring the simultaneous respective interaction of the Ras GTPase domains of the α subunits of the G_s and G_i proteins with the C2 and C1 catalytic domains of AC5.⁹ For instance, through this interaction, activation of D₂R leads to an inhibition of A_{2A}R-mediated activation of AC (Fig. 1A). In addition, the precise quaternary structure of the A_{2A}R-D₂R heterotetramer provides the frame for the ability of A_{2A}R ligands to establish allosteric interactions with D₂R ligands, not only between agonists

FIG. 1. Functional and pharmacological properties of the A_{2A}R-D₂R heterotetramer. (A) Canonical interaction, by which a D₂R agonist, such as DA, counteracts the effect of an A_{2A}R agonist, such as ADO, by a G_s-G_i antagonistic interaction at the AC level (subtype AC5). (B) Allosteric interaction, by which A_{2A}R ligands antagonistically counteract the affinity and efficacy of D₂R ligands. (C) Ligand-independent changes in the properties of A_{2A}R ligands on heteromerization with the D₂R, such as the selective decrease in the affinity of the A_{2A}R antagonist SCH442416. (D) Increased A_{2A}R signaling in the absence of the D₂R and in the absence of A_{2A}R ligands (constitutive activity) by the A_{2A}R homomer. C1 and C2, catalytic domains of AC5. White arrows indicate the direction of the intermolecular interaction. Black arrows indicate the intensity of cAMP formation (from broken to small and large solid arrows). A_{2A}R, A_{2A} receptors; AC, adenylyl cyclase; ADO, adenosine; D₂R, D₂ receptor; DA, dopamine.

but also between antagonists.¹⁰ When either an A_{2A}R agonist or an A_{2A}R antagonist binds to the orthosteric sites of the A_{2A}R homodimer within the A_{2A}R-D₂R heterotetramer, they both produce an allosteric decrease in the affinity and efficacy of D₂R ligands (Fig. 1B). On the contrary, when an A_{2A}R agonist and an A_{2A}R antagonist bind simultaneously to the two orthosteric sites of the A_{2A}R homodimer, they counteract each other's effects.¹⁰

Furthermore, there is also evidence for significant reciprocal allosteric interactions, by which D₂R agonists negatively influence A_{2A}R agonist binding.^{11,12} This would be expected by the experimentally supported evidence that indicates that, according to Kenakin, "the allosteric energy flow is reciprocal in nature; that is, if the allosteric modulator changes the affinity of the agonist, then the agonist will also change the affinity of the modulator in a like manner."¹³ Different efficacies were observed when evaluating the negative allosteric modulation of A_{2A}R agonist binding by several D₂R agonists, some of them clinically used as antiparkinsonian agents. Apomorphine produced a significantly stronger modulation of A_{2A}R agonist binding than pramipexole and rosiglitone.¹²

We have recently shown that the canonical G_s-G_i antagonistic interaction, and therefore the functional integrity of the striatal A_{2A}R-D₂R heterotetramer-AC5 complexes, depends on the integrity of very specific interactions between transmembrane domains (TMs) of the receptors, which constitute transmembrane heteromeric and homomeric interfaces, as well as between TMs of the receptors and putative TMs of AC5.² The methodology involved a peptide-interfering approach based on the use of synthetic peptides with the amino acid sequence of all TMs of both receptors and AC5. The synthetic peptides are fused to the HIV transactivator of transcription (TAT), which determines the orientation of the peptide when inserted in the plasma membrane. The TAT-TM peptides are thus screened for their ability to destabilize oligomerization detected by bimolecular fluorescence complementation (BiFC). In this technique, two complementary halves of a fluorescent molecule are separately fused to two different GPCR protomers or to a GPCR protomer and AC5. This peptide-interfering approach is providing a very powerful tool that allows the characterization of all TM interfaces involved in GPCR oligomerization.^{2,5,10}

The homomeric TM/TM interactions were the same for both the A_{2A}R and the D₂R homodimers: A TM6/TM6 interface irrespective of cotransfection with the other molecularly different receptors.² The A_{2A}R-D₂R heteromeric interface involved a symmetrical TM4-5/TM5-4 interface, but its integrity also depends on a concomitant strong electrostatic interaction between the C-terminal domain of the A_{2A}R (A_{2A}R-CT) and the intracellular end of TM5 of the D₂R. Thus, disruption of the interaction between A_{2A}R-CT and D₂R-TM5 has been shown to significantly reduce A_{2A}R-D₂R heteromerization.^{2,10,14-16} The allosteric interactions between A_{2A}R and D₂R ligands have

also been shown to depend on the integrity of the transmembrane and intracellular heteromeric interfaces.^{10,14-17}

With its reciprocal canonical and allosteric interactions, the A_{2A}R-D₂R heterotetramer acts as an integrative molecular device, which facilitates the switch between the activation and inhibition of the striatopallidal neuron⁷: A preferential A_{2A}R versus D₂R activation leads to an increase in neuronal activity determined by an A_{2A}R-mediated AC5 activation, facilitated by the allosteric counteraction of D₂R signaling.⁷ Conversely, a preferential D₂R over A_{2A}R activation leads to a decrease in neuronal activity determined by a D₂R-mediated activation of phospholipase C and facilitated by the canonical and reciprocal allosteric interactions, thus switching off the A_{2A}R-mediated AC5 activation.⁷ Indeed, the activation of the striatopallidal neuron leads to withdrawal behaviors and, when intense, to motor arrest and catalepsy. On the contrary, striatopallidal neuron inhibition leads to psychomotor activation.^{7,18}

Functional Role of the Presynaptic Striatal A₁R-A_{2A}R Heterotetramer

Adenosine plays an important modulatory role driving the function of corticostriatal terminals. This control is mediated by adenosine A₁R and A_{2A}R, which establish intermolecular interactions forming A₁R-A_{2A}R heterotetramers.^{5,19} Interestingly, these heteromers work as an adenosine concentration-dependent switch, which determines the opposite effects of adenosine on glutamate release depending on a predominant A₁R or A_{2A}R activation within the heteromer.^{19,20} Importantly, the affinity of adenosine is higher for A₁R than for A_{2A}R. Therefore, low concentrations of adenosine primarily activate A₁R, which induces an inhibition of glutamate release. On the contrary, on high concentrations of adenosine, such as those obtained under strong glutamatergic transmission and the consequent neuronal and glial ATP release and conversion of ATP on adenosine,²¹ the simultaneous activation of A_{2A}R leads to an allosteric modulation within the heteromer, with a reduction of affinity and efficacy of adenosine for the A₁R.^{5,19} Under these conditions A_{2A}R signaling prevails and an adenosine-mediated facilitatory glutamate release is achieved. Importantly, the molecular mechanisms of these interactions are beginning to be understood and they are related to the specific quaternary structure of the A₁R-A_{2A}R heterotetramer.⁵ According to the reciprocal nature of allosterism (see the Functional Role of the Postsynaptic Striatal A_{2A}R-D₂R Heterotetramer section),¹³ we could also expect that A₁R ligands allosterically modulate A_{2A}R ligands in the A₁R-A_{2A}R heterotetramer, but this needs still to be demonstrated.

The A₁R is a classical G_i-coupled receptor. However, different to the A_{2A}R-D₂R heterotetramer, the A₁R-A_{2A}R heterotetramer does not sustain a canonical G_s-G_i antagonistic interaction at the AC level and the A_{2A}R-mediated AC activation is unopposed by A₁R activation.⁵

This correlates with the A_{2A}R-mediated striatal glutamate release unopposed by A₁R activation.¹⁹ Particularly striking was the finding that deletion of A_{2A}R-CT enables the canonical G_s-G_i antagonistic interaction in cells coexpressing A₁R and A_{2A}R.⁵ Different from the A_{2A}R-D₂R heterotetramer (see the Functional Role of the Postsynaptic Striatal A_{2A}R-D₂R Heterotetramer section), A_{2A}R-CT deletion did not disrupt A₁R-A_{2A}R heteromerization, indicating its lack of participation on the stabilization of the quaternary structure of the A₁R-A_{2A}R heterotetramer.⁵ Interestingly, when compared to the A_{2A}R-D₂R heterotetramer, different TM/TM homomeric and heteromeric interfaces were observed using TAT-TM peptides in BiFC experiments: TM4–5/TM5–4 homomeric interfaces for both A_{2A}R and A₁R homomers and a TM5–6/TM6–5 for the A_{2A}R-A₁R heteromeric interface.⁵ Molecular dynamics computational analysis already predicts that GPCR homodimers display several possible homodimeric TM interfaces.²² Our studies indicate that the preferred homomeric interfaces in a GPCR heterotetramer are determined by the heteromeric partner.

An important implication from the study of the role of GPCR heteromers is that they are required for the functional expression of G_i-coupled receptors in the modulation of certain subtypes of AC. More specifically, those AC isoforms can be inhibited by the α subunits of G_{i/o} proteins, including AC1, AC5, and AC6.²³ Artificially, G_i-coupled receptors can inhibit forskolin-induced AC activation, but we have demonstrated that to inhibit a G_s-coupled receptor-mediated AC5 activation, they need to be part of a GPCR heterotetramer. This has been so far shown for the A_{2A}R-D₂R, A₁R-D₁R, and D₁R-D₃R heterotetramers, where destabilization of their heteromeric interface leads to the disruption of the canonical G_s-G_i antagonistic interaction at the AC level.^{2,24,25} The A₁R-A_{2A}R heterotetramer represents a particular case, since, because of interference with the A_{2A}R-CT, the G_i-coupled A₁R cannot counteract the G_s-coupled A_{2A}R-mediated AC activation.⁵ Nevertheless, it is quite well established that the main mechanism involved in the modulation of neurotransmitter release by G_i-coupled receptors, including A₁R, is by a decrease in the probability of neurotransmitter release through a direct inhibition of G_i protein $\beta\gamma$ subunits of N- and P/Q-type voltage-dependent calcium channels.^{26,27} On the contrary, G_s protein-coupled receptors can produce and increase in the probability of neurotransmitter release by an AC-cAMP-PKA-dependent mechanism.^{28,29} Therefore, the A₁R-A_{2A}R heterotetramer is particularly designed to inhibit and stimulate glutamate release by an AC-independent and AC-dependent mechanism, respectively.

In addition to providing the frame for allosterical interactions between ligands binding to their orthosteric sites and interactions at the effector level (canonical G_s-G_i antagonistic interaction), heteromerization can lead to changes in the properties of specific ligands for one of

the protomers, independent of other ligands binding to the other protomer (Fig. 1C). Indeed, the proof of concept came from experiments in transfected mammalian cells, where the potencies of different selective A_{2A}R antagonists at binding to the A_{2A}R alone or when coexpressed either with D₂R or A₁R were compared.³⁰ Interestingly, the most dramatic finding was that the A_{2A}R antagonist SCH442416 showed a selective low affinity for the A_{2A}R when coexpressed with D₂R compared with its affinity for the A_{2A}R alone or coexpressed with A₁R (Fig. 1C).³⁰ More specifically, SCH442416 showed a pronounced negative cooperativity of its binding to the A_{2A}R homodimer within the A_{2A}R-D₂R heterotetramer.^{7,30}

This was further demonstrated in striatal preparations from mice with conditional striatal D₂R-KO, which, in contrast to wild-type mice, did not show the binding negative cooperativity of SCH442416.⁷ As expected, SCH442416 showed a dissociation on its ability to produce locomotor activity (which should depend on its binding to the postsynaptic A_{2A}R-D₂R heterotetramer) versus its ability to inhibit electrically and optogenetically induced striatal glutamate release (which should depend on its binding to the presynaptic A₁R-A_{2A}R heterotetramer).^{7,30} Both in rats and mice, higher systemic doses were necessary to produce locomotion than those necessary to produce inhibition of glutamate release.^{7,30} Importantly, the preferential presynaptic profile of SCH442416 was confirmed by further studies by other research groups^{31,32} and was suggested to provide a therapeutic approach for conditions with increased corticostriatal transmission, such as cannabinoid use disorder.³³

The same mechanism has recently been reported for the selective significant decrease in potency of the μ -opioid receptor agonist methadone, compared with morphine and fentanyl, in the μ -opioid-galanin Gal₁ receptor heteromer.³⁴ Since these heteromers are selectively localized in the ventral tegmental area (localization of cell bodies of dopaminergic cells involved in the processing of natural rewards), this could explain a selective dissociation of the clinical therapeutic versus euphoric/rewarding effects of methadone compared with other opioids.³⁴ Therefore, changes in the pharmacological properties of ligands induced by receptor heteromerization should constitute a very important strategy in the search for new therapeutic agents with further selectivity for their therapeutic versus side, unwanted effects.

Neuroprotective Role of the Postsynaptic Striatal A_{2A}R-D₂R Heterotetramer: Impact on D₂R Signaling in PD

PD, the second most common age-related neurodegenerative disorder, is characterized by a progressive loss of nigrostriatal dopaminergic cells, which affects ~1% of individuals older than 60 years.³⁵ PD symptoms include resting tremor, rigidity, bradykinesia, or postural instability

and, in advanced stages, cognitive dysfunction and dementia.³⁶ Only 10–15% of PD cases classify as early-onset familial PD (recognized as having a first-degree affected family member),³⁷ while the remaining cases are idiopathic, thus indicating a key role for nongenetic and environmental factors in PD pathogenesis. Indeed, exposure to environmental toxins (such as pesticides, solvents, heavy metals, and other pollutants) can cause dopaminergic cell death.³⁸ Furthermore, oxidative stress, mitochondrial dysfunction, and inflammation play key roles in PD pathophysiology.^{39,40} Accordingly, PD treatments are mainly based on the use of prodopaminergic drugs, intending to restore neurotransmitter deficiency on the loss of nigrostriatal dopaminergic neurons.⁴¹ However, this pharmacological treatment also presents many undesired effects, specially on chronic consumption. Thus, although L-DOPA has been proved very efficacious in the treatment of PD symptoms, its long-term treatment tends to lose efficacy and also to induce severe collateral motor effects (dyskinesia and rigidity) and psychiatric symptoms.⁴² Interestingly, most epidemiological studies support a protective benefit of habitual drinking of caffeinated beverages,^{43–45} thus suggesting a neuroprotective role of adenosine receptors in PD.

The striatum plays a central role in PD pathophysiology. Indeed, dopamine depletion throughout the course of PD progression is followed by a substantial cellular and molecular striatal remodeling. Postmortem studies using PD necropsies revealed a reduced dendritic length and spine density in striatal GABAergic neurons.⁴⁶ The caudal part of the putamen, where PD-associated dopamine deficits are more pronounced, shows the highest reduction in spine density.⁴⁷ In addition, some biochemical alterations in postmortem PD brains indicate a protein homeostasis dysregulation,⁴⁸ which includes the expected downregulation of the rate-limiting enzyme in dopamine synthesis tyrosine hydroxylase⁴⁹ and the existence of the intracytoplasmic α -synuclein fibrillar aggregates that constitute the PD histopathological hallmark, the Lewy body.⁴⁸ The levels of several neurotransmitters and the expression of their receptors have been found to be altered, not without some discrepancies.⁴⁸ Thus, while the levels of dopamine are reduced in the whole striatum (caudate, nucleus accumbens, and putamen) and globus pallidus, they were not found to be altered in the subthalamic nucleus, thalamus, and substantia nigra.⁵⁰ Divergences were found when measuring the striatal D₂R density, with some studies reporting that D₂R is upregulated,⁵¹ downregulated,⁵² or unaltered.^{53–55} Interestingly, the adenosinergic system has also been found dysregulated in PD, with no changes in adenosine levels,⁵⁶ but a significant increase in A_{2A}R density.^{55,57} Therefore, it is not unreasonable to expect that altered dopamine and adenosine levels and D₂R and A_{2A}R densities within the striatum of PD subjects should determine alterations in the A_{2A}R-D₂R heteromer composition and function, thus impacting PD pathophysiology.

Indeed, we could demonstrate the existence of A_{2A}R-D₂R heteromers in native tissue by using a multimethodological approach (i.e., immunoelectron microscopy, proximity ligation assay, and time-resolved fluorescent resonance energy transfer) in the striatum of control and unilateral 6-OHDA-lesioned rats (a widely used animal model of PD).⁵⁸ Interestingly, a significant reduction of the A_{2A}R-D₂R heteromer content was observed in the 6-OHDA-denervated striatum.⁵⁸ The striatal A_{2A}R-D₂R heteromer disruption observed in this PD animal model might then constitute a neuroadaptive response associated with dopaminergic denervation in PD. Future efforts should determine similar changes in the A_{2A}R-D₂R heteromer status in postmortem caudate-putamen from PD subjects. Indeed, establishing the A_{2A}R-D₂R heteromer status in PD could determine the design of selective combined pharmacotherapeutic strategies restoring the unbalanced A_{2A}R-D₂R heteromer function potentially associated with PD.

In the same PD animal model, the A_{2A}R demonstrated a significant constitutive activity and this uncontrolled activity was blocked by caffeine and other prototypic A_{2A}R inverse agonists.⁵⁹ Therefore, we assumed that the striatal A_{2A}R-D₂R heteromer disruption observed in this PD animal model, and the expected loss of the negative canonical and allosteric control by the D₂R was behind the gain of A_{2A}R constitutive activity, which could be involved in the striatal neurodegeneration of PD (Fig. 1D). This provides a rationale for the use of A_{2A}R antagonists in PD and for fostering the research of mechanisms that could restore an unbalanced expression of A_{2A}R homomers versus A_{2A}R-D₂R heteromers in this disease.

Neuroprotective Role of the Presynaptic Striatal A₁R-A_{2A}R Heterotetramer: Reduction of A₁R Signaling in RLS

RLS is a very common neurological disorder, characterized by periodic, rest-induced, mostly nocturnal, movement-responsive urge to move the legs or periodic leg movements during sleep (PLMS) and hyperarousal.^{60–63} The deficits of sensorimotor integration that promote PLMS and hyperarousal are interrelated, and adenosine seems to be a very important pathogenetic link. Several preclinical and clinical data indicate the existence of a hypoadenosinergic state secondary to brain iron deficiency (BID) as an initial pathogenetic mechanism in RLS.^{63–65} It has been demonstrated that BID in the experimental animal leads to a generalized downregulation of A₁R.⁶⁶ A₁R downregulation in the cortex and in the areas of origin of the ascending arousal systems could explain the hyperarousal.^{63,64} On the contrary, A₁R downregulation in the striatum could explain an increased sensitivity of corticostriatal terminals, which has recently proposed to be a main mechanism responsible for the deficits of sensorimotor

integration that promote PLMS.^{65,67} In fact, corticostriatal glutamatergic terminals are targets for the drugs most often prescribed in RLS, the dopamine receptor agonists pramipexole and ropinirole and the $\alpha_2\delta$ -ligand gabapentin.⁶⁷

The possible key role of A₁R downregulation in corticostriatal glutamatergic terminals in the sensorimotor symptomatology of RLS was significantly supported by preclinical and clinical experiments. First, we predicted that equilibrative nucleoside transporter inhibitors, by increasing the striatal extracellular levels of adenosine (which would facilitate the binding probability of adenosine to the lower expressed A₁R), could provide a new therapeutic approach for RLS. In fact, we recently reported encouraging results with the nonselective ENT1/ENT2 inhibitor dipyridamole in an open trial with RLS patients.⁶⁸ At the preclinical level, as predicted, an A₁R antagonist produced hypersensitivity of corticostriatal terminals and reduced the frequency of optogenetic stimulation necessary to induce corticostriatal glutamate release.⁶⁵ Furthermore, dipyridamole, by increasing the striatal extracellular concentration of adenosine and the activation of presynaptic A₁R, was able to counteract optogenetic-induced glutamate release in both naive rats and in rats with BID.⁶⁵ Finally, as we also expected, this effect of dipyridamole was counteracted by the A₁R antagonist.⁶⁵

Similar to what seems to occur with postsynaptic A_{2A}R in PD, the pathogenesis of RLS could involve a change in the stoichiometry of A_{2A}R and A₁R forming and not forming heterotetramers. A₁R downregulation would mean a relative increase in A_{2A}R/A₁R expression ratio, which should lead to a relative decrease of A₁R-A_{2A}R heteromers and a relative increase of A_{2A}R not forming heteromers. Unopposed by the A₁R signaling in the heterotetramer, the relative increase in A_{2A}R expression and constitutive activity should be indirectly responsible for the A₁R downregulation-mediated increased sensitivity of the corticostriatal glutamatergic terminals. This would predict that A_{2A}R antagonists that target A_{2A}R not forming heteromers could also be of therapeutic use in RLS. In fact, we have previously shown that the nonselective pre/postsynaptic A_{2A}R antagonist MSX-3 significantly counteracts optogenetically induced corticostriatal glutamate release.⁶⁹

Adenosine A₁R-A_{2A}R heteromers have also been demonstrated in cortical astrocytic cultures, where they modulate GABA transport by GAT-1 and GAT-3 transporters.⁷⁰ The same as the presynaptic striatal A₁R-A_{2A}R heteromer, it acts as an adenosine concentration-dependent switch, which, in this case, determines the opposite effects of adenosine on GABA transport depending on a predominant A₁R or A_{2A}R activation within the heteromer.⁷⁰ If also functionally present in the striatum, it could be involved in the pathogenesis of RLS. A reduction of A₁R density would imply an increased A_{2A}R-mediated GABA transport and, there-

fore, a reduction in the extracellular levels of the inhibitory neurotransmitter GABA.

Concluding Remarks

GPCR heteromers are changing classical views of GPCR physiology and pharmacology. First, they are providing a better understanding of interactions between different neurotransmitters and exogenous ligands, based on their ability to convey canonical interactions at the effector level and allosteric interactions between endogenous and exogenous ligands. This, for instance, provides the rationale for the use of A_{2A}R antagonists in PD, which increase the therapeutic index of L-DOPA.^{71,72} Second, GPCR heteromerization determines potential pharmacodynamic differences between exogenous compounds, such as the A_{2A}R antagonist SCH442416, with its preferential binding to the presynaptic A₁R-A_{2A}R versus postsynaptic A_{2A}R-D₂R heterotetramers. This provides the rationale for the use of SCH442416-like compounds in conditions with an excess of corticostriatal signaling, for instance, in substance use disorders.^{31–33,73} Finally, we believe this review provides sufficient background that supports a pathogenetic role of postsynaptic striatal A_{2A}R-D₂R heterotetramers in PD and presynaptic A₁R-A_{2A}R heterotetramers in RLS, based on their decreased expression versus the expression of the Gs-coupled A_{2A}R not forming heteromers, which, free from the antagonistic control of the Gi-coupled D₂R and A₁R, facilitate an increased neuronal activation and presynaptic glutamate release, respectively. In both cases, A_{2A}R antagonists that could preferentially target A_{2A}R not forming heteromers could constitute a successful therapeutic strategy.

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Author Disclosure Statement

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Address correspondence to:
Sergi Ferré, MD, PhD
Integrative Neurobiology Section
National Institute on Drug Abuse
Intramural Research Program
National Institutes of Health
Triad Technology Building
333 Cassell Drive
Baltimore, MD 21224
E-mail: sferre@intra.nida.nih.gov