PERSPECTIVE

Adenosine A_{2A}-dopamine D_2 receptor heteromers operate striatal function: impact on Parkinson's disease pharmacotherapeutics

The basal ganglia (BG) assemble a series of deep gray matter structures forming recurrent loops that include the cortex and thalamus, and that participate in the regulation of a plethora of brain functions, including elicitation and learning of reward-and aversive stimuli-associated behaviors, motor activity control and sensorimotor gating (Bromberg-Martin et al., 2010). The striatum is the main input BG structure, thus it receives cortical glutamatergic projections from widespread areas of cortex and projects into other BG nuclei, including globus pallidus pars externa and the BG outputsglobus pallidus pars interna and substantia nigra pars reticulata. On the other hand, the substantia nigra pars compacta-ventral tegmental area (SNpc-VTA) modulates cortical-BG-thalamic circuits by means of dopaminergic innervation of the striatum. Interestingly, the main population of striatal neurons, the medium spiny neurons (MSNs), provide the origin of two different striatal efferent pathways, the direct and indirect pathways (Schiffmann et al., 2007). Both project to the BG outputs, and the direct pathway also projects to brainstem, to the SNpc-VTA. The MSN originating these two pathways are characterized by the differential expression of several key genes. Thus, while MSNs from the direct pathway (direct MSNs) express dopamine D_1 receptors (D_1R) and contain the neuropeptides dynorphin and substance P, indirect MSNs express dopamine D_2 receptors (D_2R) and contain the neuropeptide enkephalin (Fuxe et al., 2007; Schiffmann et al., 2007). Striatal dopamine from SNpc-VTA projections potentiates direct and inhibits indirect pathway MSN, which leads to a net inhibition of thalamocortical areas.

Interestingly, D_1Rs and D_2Rs, respectively localized in the direct and indirect MSNs, are functionality tuned by direct receptor-receptor interactions (*i.e.*, heteromerization) established with other endogenously expressed G protein-coupled receptors, for instance adenosine, metabotropic glutamate and cannabinoid receptors, among others. In this way, these heteromers may positively or negatively regulate D_1R and/or D_2R activation, a fact that may ultimately constitute a very complex scenario in which the control of BG functioning is regulated by a myriad of complex interactions between different neurotrasmistters (Fuxe et al., 2007). From these receptor-receptor complexes, one of the most studied is the one formed by D_2R and the adenosine A_{2A} receptor ($A_{2A}R$) in the indirect MSN. In such way, it has been shown that reciprocal antagonistic interactions occur within the $A_{2A}R$-D_2R heteromer (Fuxe et al., 2007). Thus, an *allosteric* interaction was initially described, by which $A_{2A}R$ ligands decrease both the affinity and intrinsic efficacy of D_2R ligands (Ferré et al., 2016). And, more recently, an opposite interaction was described, by which D_2R agonists decrease the binding of $A_{2A}R$ ligands (Fernández-Dueñas et al., 2013) (**Figure 1**). In addition, a strong antagonistic interaction (namely *canonical*) at the adenylyl cyclase (AC) level has been described, which depends on the ability of D_2R-mediated Gi activation to inhibit Gs activation elicited by $A_{2A}R$ activation (Ferré et al., 2016) (**Figure 1**). Interestingly, the occurrence of both kinds of reciprocal antagonistic interactions (*canonical* and *allosteric*) led to conjecture the existence of different populations of receptors: forming and non-forming oligomers. Accordingly, in such a model $A_{2A}R$-D_2R heterodimers would preferentially allow the *allosteric* interaction, while the formation of D_2R-D_2R or $A_{2A}R$-$A_{2A}R$ homodimers would be mainly responsible of the *canonical* interaction. Alternative-

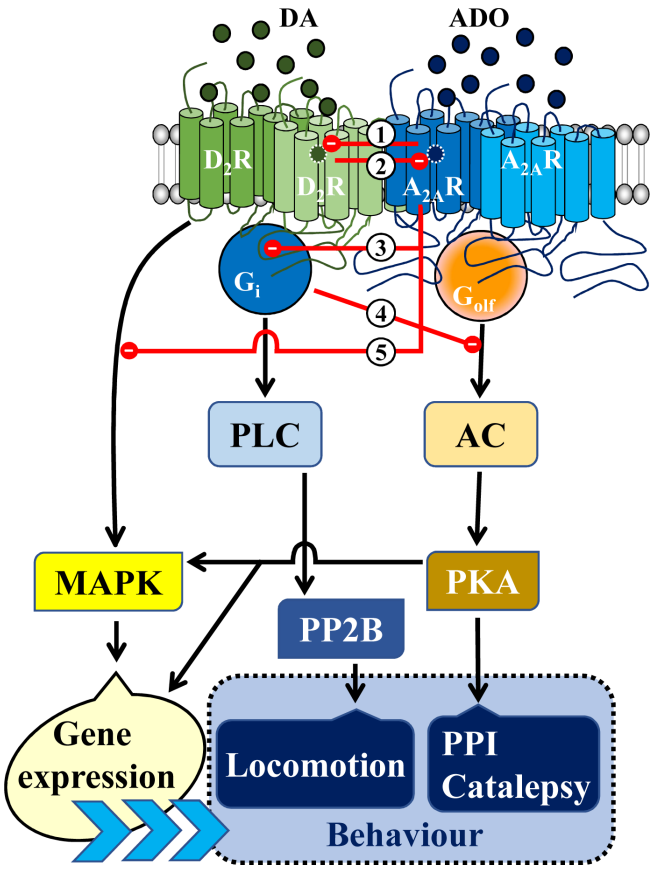

Figure 1 Scheme showing the proposed striatal adenosine A_{2A} receptor ($A_{2A}R$)-dopamine D_2 receptors (D_2R) heteromer-dependent modulation of basal ganglia behavioral outputs.
The heterotetrameric structure of the $A_{2A}R$-D_2R heteromer allows a multimodal functional interplay between adenosine (ADO) and dopamine (DA). Thus, ADO decreases D_2R agonist affinity through an *allosteric* interaction (1) and it reduces D_2R signaling through G protein-dependent (3) and G protein-independent (5) pathways. Conversely, DA activation of D_2R decreases $A_{2A}R$ agonist affinity through an *allosteric* interaction (2) and it inhibits $A_{2A}R$ agonist-mediated activation of adenylyl cyclase (AC), by means of the antagonistic Gs-Gi *canonical* interaction at the AC level (4). When uninterrupted by the canonical interaction, $A_{2A}R$ signals through activation of AC and protein kinase A (PKA), resulting in an increasein the excitability of indirect medium spiny neurons (MSNs), which facilitates prepulse inhibition (PPI) and promotes catalepsy. When uninterrupted by the allostericinteraction, D_2R signals through phospholipase C (PLC), which leads to activation of protein phosphatase 2B (PP2B) and a decrease in the excitability of indirect MSNs, which facilitates locomotion. Interestingly, $A_{2A}R$ and D_2R activation can also modify gene expression through different mechanisms, including G protein-dependent and independent mitogen activated protein kinase (MAPK) activation, respectively.

ly, the recent hypothesis of the formation of heterotetrameric structures could explain the simultaneous existence of both types of receptor-receptor interactions (Bonaventura et al., 2015; Ferré et al., 2016) (**Figure 1**). In fact, most existing experimental data on the biochemical and behavioral effects of $A_{2A}R$ and D_2R ligands can be explained in the frame of one main population of striatal A_2R forming $A_{2A}R$-D_2R heteromers (Taura et al., 2017). However, it is important to note that although the existence of the direct $A_{2A}R$-D_2R interaction has been widely accepted for many years, the demonstration of its existence in native tissue was only made recently. First, co-immunoprecipitation experiments and immunoelectron microscopy studies showed the association and co-distribution of D_2R and $A_{2A}R$ in rat striatum (Cabello et al., 2009). Next, it was possible to ascertain the close proximity of D_2R and $A_{2A}R$ by means of the proximity ligation assay (PLA) in mice and sheep striatum (Trifilieff et al., 2011; Bonaventura et al., 2015), in which it was also observed the ability of a synthetic peptide to specifically disrupt the PLA in sheep striatum (Bonaventura et al., 2015). Finally, the clearest demonstration of $A_{2A}R$-D_2R heteromers in striatal tissue came from a complementary approach using immunoelectron microscopy, PLA and time-resolved Fluorescence Resonance Energy Transfer (TR-FRET) with specific fluorescence ligands in rats (Fernández-Dueñas et al., 2015).

At this point, we can clearly state that striatal D_2Rs form homo- and hetero-complexes with other receptors, for instance the $A_{2A}R$, and that the above-described receptor-receptor interactions may lead to a fine-tuning regulation of BG function. However, apart from the obvious interest of revealing the functional role of $A_{2A}R$-D_2R heteromers in these brain areas, a high effort is being dedicated to elucidating their role in the pathophysiology and pharmacotherapeutics of Parkinson's disease (PD). PD is a neurodegenerative disease that affects approximately 1% over the age of 60, which turns to 5% in subjects up to 85 years.The etiology of this pathology is not well determined, and both genetic and environmental factors may be involved. Conversely, it is well-accepted that the main symptoms of PD (bradykinesia, rigidity, resting tremor and posture instability) appear when a large proportion of dopaminergic fibers from the SNpc projecting into the striatum are lost. Therefore, the main PD treatment consists of trying to restore dopamine levels by administrating dopamine-based drugs, from which the precursor L-DOPA has been the most extensively used since the seventies. However, chronic treatment with L-DOPA invariably leads to the occurrence of severe side-effects, such as dyskinesia, thus a lot of efforts are being directed to find out novel non-dopaminergic-based drugs with a lower incidence of these side effects. One of these new approaches consists of the development of selective $A_{2A}R$ antagonists (for review, see Vallano et al., 2011), whose mode of action may consist off acilitating D_2R functionor by an independent effect on $A_{2A}R$ not interacting with D_2R. Thus, as recently addressed in (Taura et al., 2017), there is evidence for an upregulation of $A_{2A}R$ upon dopamine denervation, which should lead to the presence of a significant population of $A_{2A}R$ not forming heteromers with D_2R in PD. Accordingly, either by the direct blockade of $A_{2A}R$ function, which may be over-activatedin PD, or by precluding the negative *allosteric* interaction within the $A_{2A}R$-D_2R heteromer, $A_{2A}R$ antagonists have been demonstrated to exert beneficial effects in PD animal models and also in PD patients (Vallano et al., 2011).

Noteworthy, to date, just one $A_{2A}R$ antagonist has been approved for human use and introduced into clinics (Vallano et al., 2011); while others have not survived clinical trials (for several reasons) or are still under investigation. In view of that, and within the $A_{2A}R$-D_2R heteromerization context, it would seem likely to revisit the pharmacological strategy used to select $A_{2A}R$-based drugs for PD therapeutics. Accordingly, we propose that it would be important to characterize the *in vivo* efficacy of the putative effects of $A_{2A}R$ ligands, considered to be implemented in PD therapeutics, on the striatal $A_{2A}R$ forming and not forming heteromers with D_2R. An example of this kind of approach is the recent study we set to readdress the *in vivo* pharmacological properties of the $A_{2A}R$ antagonist SCH442416 (Taura et al., 2017). Of note, we took advantage of genetic manipulation, using wild-type and D_2R or $A_{2A}R$ deficient mice ($D_2R^{-/-}$ and $A_{2A}R^{-/-}$, respectively), in order to dissect the role of $A_{2A}R$-D_2R heteromerization on the behavioral effects of this ligand. First, a significant but partial reduction of activity was observed when evaluating spontaneous locomotor activity in $D_2R^{-/-}$ or $A_{2A}R^{-/-}$ mice, thus pointing to the existence of neuroadaptations that counteract the respective loss of D_2R- and $A_{2A}R$-mediated tonic stimulation and inhibition of psychomotor activity. When examining the effects of SCH442416-mediated activation of locomotion, we observed a substantial diminished effect in $D_2R^{-/-}$ mice, which would be consistent with a dependence on the *allosteric* interaction within the $A_{2A}R$-D_2R heteromer (Taura et al., 2017). On the other hand, our data also provided further support to the importance of $A_{2A}R$ mediation in the behavioral effects secondary to the activation or interruption of D_2R signaling, such as inhibition of sensorimotor gating or catalepsy, respectively, consistent with a dependence on the *canonical* interaction within the $A_{2A}R$-D_2R heteromer. Thus, SCH442416 counteracted the inhibitory effect the D_2R agonist sumanirole on prepulse inhibition (PPI) and the cataleptic effect of the D_2R antagonist haloperidol. On the other hand, the $A_{2A}R$ agonist CGS21680-induced catalepsy was completely counteracted by the genetic blockade of $A_{2A}R$ and only a partial counteraction was observed in $D_2R^{-/-}$ mice (Taura et al., 2017). Nevertheless, SCH442416 can counteract CGS21680-mediated catalepsy in $D_2R^{-/-}$ mice (unpublished data), indicating that this can be used as a method to evaluate the potency and efficacy of $A_{2A}R$ antagonists on $A_{2A}R$ not forming heteromers with D_2R.

We have recently proposed a heuristic model of the operation of the $A_{2A}R$ and D_2R in the indirect MSN that integrates the information available in the literature together with our more recent behavioral results (see Taura et al., 2017 and **Figure 1**). The model proposes that D_2R mainly signals by activating phospholipase C (PLC) through α β γ subunit-dependent mechanism, thus activating the Ca^{2+}/calmodulin-dependent protein phosphate calmodulin (PP2B), which leads to enhancing locomotor activity (**Figure 1**). Similarly, $A_{2A}R$ mainly signals through activation of AC and protein kinase A (PKA), which facilitates PPI and catalepsy (**Figure 1**). In addition, D_2R and $A_{2A}R$ activation also modify gene expression by G protein-independent or dependent mitogen-activated protein kinase (MAPK) activation (**Figure 1**). In the frame of the $A_{2A}R$-D_2R heteromer, it seems clear

that these signaling cascades are determined by the receptor that is predominantly activated and the consequent predominant interaction, *allosteric* or *canonical* (Taura et al., 2017) (**Figure 1**). Obviously, these interactions should be absent in the frame of the $A_{2A}R$ not forming heteromers, which should be independent from D_2R signaling.

Overall, the accumulation of data since the early 1990's regarding the interactions between D_2R and $A_{2A}R$ has prompted to a more comprehensive representation of the functioning of the $A_{2A}R$-D_2R heteromer in the BG context. However, although the formation of $A_{2A}R$-D_2R heteromers in native tissue and their significant functional and pharmacological significance is becoming generally accepted, we need to determine their role in the pathophysiology and treatment of PD and other neuropsychiatric disorders, particularly in view of the possible changes in the stoichiometry of $A_{2A}R$ and D_2R, when attempting to develop novel pharmacological tools such as new $A_{2A}R$ antagonists.

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***Comments to authors:** *In the present manuscript Fernández-Dueñas et al. reviewed the current state of knowledge of the interaction between the D_2 dopamine receptor and A_{2A} adenosine receptor, and how this interaction can control the activity of striatum neurons in control and pathological conditions such as Parkinson's disease. The article is well written, clear, interesting to the research field and possibly for the development of future pharmacotherapy of Parkinson`s disease.*

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