**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 outputglobus pallidus pars interna and substantia nigra pars reticulata. On the other hand, the substantia nigra pars compacta-ventral tegmental area (SNpc-VTA) modulates cortical-BGthalamic 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$_1$R) and contain the neuropeptides dynorphin and substance P, indirect MSNs express dopamine D$_2$ receptors (D$_2$R) 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 thalamo-cortical areas.

Interestingly, D$_1$Rs and D$_2$Rs, 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$_1$R and/or D$_2$R 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 neurotransmitters (Fuxe et al., 2007). From these receptor-receptor complexes, one of the most studied is the one formed by D$_1$R 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$_2$R 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$_2$R ligands (Ferré et al., 2016). And, more recently, an opposite interaction was described, by which D$_2$R 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$_2$R-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$_2$R heterodimers would preferentially allow the allosteric interaction, while the formation of D$_2$R-D$_2$R or A$_{2A}$R-A$_{2A}$R homodimers would be mainly responsible of the canonical interaction. Alternative-
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 A2A-R and D2-R ligands can be explained in the frame of one main population of striatal A2A-R forming A2A-R-D2-R heteromers (Taura et al., 2017). However, it is important to note that although the existence of the direct A2A-R-D2-R 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 D2-R and A2A-R in rat striatum (Cabello et al., 2009). Next, it was possible to ascertain the close proximity of D2-R and A2A-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 A2A-R-D2-R 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 D2-Rs form homo- and hetero-complexes with other receptors, for instance the A2A-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 A2A-R-D2-R 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 A2A-R antagonists (for review, see Vallano et al., 2011), whose mode of action may consist of facilitating D2-R function by an independent effect on A2A-R not interacting with D2-R. Thus, as recently addressed in (Taura et al., 2017), there is evidence for an upregulation of A2A-R upon dopamine denervation, which should lead to the presence of a significant population of A2A-R not forming heteromers with D2-R in PD. Accordingly, either by the direct blockade of A2A-R function, which may be over-activated in PD, or by precluding the negative allosteric interaction within the A2A-R-D2-R heteromer, A2A-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 A2A-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 A2A-R-D2-R heteromerization context, it would seem likely to revisit the pharmacological strategy used to select A2A-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 A2A-R ligands, considered to be implemented in PD therapeutics, on the striatal A2A-R forming and not forming heteromers with D2-R. An example of this kind of approach is the recent study we set to readdress the in vivo pharmacological properties of the A2A-R antagonist SCH442416 (Taura et al., 2017). Of note, we took advantage of genetic manipulation, using wild-type and D2-R or A2A-R deficient mice (D2-R–/– and A2A-R–/–, respectively), in order to dissect the role of A2A-R-D2-R heteromerization on the behavioral effects of this ligand. First, a significant but partial reduction of activity was observed when evaluating spontaneous locomotor activity in D2-R–/– or A2A-R–/– mice, thus pointing to the existence of neuroadaptations that counteract the respective loss of D2-R and A2A-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 D2-R–/– mice, which would be consistent with a dependence on the allosteric interaction within the A2A-R-D2-R heteromer (Taura et al., 2017). On the other hand, our data also provided further support to the importance of A2A-R mediation in the behavioral effects secondary to the activation or interruption of D2-R signaling, such as inhibition of sensorimotor gating or catalepsy, respectively, consistent with a dependence on the canonical interaction within the A2A-R-D2-R heteromer. Thus, SCH442416 counteracted the inhibitory effect the D2-R agonist sumanirole on prepulse inhibition (PPI) and the cataleptic effect of the D2-R agonist haloperidol. On the other hand, the A2A-R agonist CGS21680-induced catalepsy was completely counteracted by the genetic blockade of A2A-R and only a partial counteraction was observed in D2-R–/– mice (Taura et al., 2017). Nevertheless, SCH442416 can counteract CGS21680-mediated catalepsy in D2-R–/– mice (unpublished data), indicating that this can be used as a method to evaluate the potency and efficacy of A2A-R antagonists on A2A-R not forming heteromers with D2-R.

We have recently proposed a heuristic model of the operation of the A2A-R and D2-R 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 D2-R mainly signals by activating phospholipase C (PLC) through αβγ subunit-dependent mechanism, thus activating the Ca2+/calmodulin-dependent protein phosphatase calmodulin (PP2B), which leads to enhancing locomotor activity (Figure 1). Similarly, A2A-R mainly signals through activation of AC and protein kinase A (PKA), which facilitates PPI and catalepsy (Figure 1). In addition, D2-R and A2A-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 A2A-R-D2-R 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 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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Victor Fernández-Dueñas, Sergi Ferré, Francisco Ciruela* Unitat de Farmacologia, DepartamentPatologia i Terapeutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L’Hospital de Llobregat, Barcelona, Spain (Fernández-Dueñas V, Ciruela F)

Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain (Fernández-Dueñas V, Ciruela F)

Integrative Neurobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA (Ferré S)*

*Correspondence to: Francisco Ciruela, fciruela@ub.edu.

orcid: 0000-0003-0832-3739 (Francisco Ciruela)

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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.

References

Bonaventura J, Navarro G, Casadó-Ánguera V, Azad K, Rea W, Moreno E, Brugarolas M, Mallol J, Canela EI, Lluís C, Cortés A, Volkow ND, Schiffrin SN, Ferré S, Casadó V (2015) Allosteric interactions between agonists and antagonists within the adenosine A2A receptor-dopamine D2 receptor heterotetramer. Proc Natl Acad Sci U S A 112:E3609-3618.

Bromberg-Martín ES, Hikosaka O, Nakamura K (2010) Coding of task reward value in the dorsal raphe nucleus. J Neurosci 30:6262-6272.

Cabello N, Gandía J, Berrutellic DC, Watanabe M, Lluís C, Franco R, Ferré S, Luján R, Ciruela F (2009) Metabotropic glutamate type 5, dopamine D2 and adenosine A2A receptors form higher-order oligomers in living cells. J Neurochem 109:1497-1507.

Fernández-Dueñas V, Gómez-Soler M, Morató X, Núñez F, Das A, Kumar TS, Jaumà S, Jakobsen KA, Ciruela F (2013) Dopamine D2 receptor-mediated modulation of adenosine A2A receptor agonist binding within the A2A/D2R oligomer framework. Neurochem Int 63:42-46.

Fernández-Dueñas V, Taura JJ, Cotet M, Gómez-Soler M, López-Cano M, Ledent C, Watanabe M, Trinquet E, Pin JP, Luján R, Durroux T, Ciruela F (2015) Untangling dopamine-adenosine receptor-receptor assembly in experimental parkinsonism in rats. Dis Model Mech 8:57-63.

Ferré S, Bonaventura J, Tomasi D, Navarro G, Moreno E, Cortés A, Lluís C, Casado V, Volkow ND (2016) Allosteric mechanisms within the adenosine $A_2A_R$-dopamine $D_2_R$ receptor heterotetramer. Neuropharmacology 104:154-160.

Fuxe K, Ferré S, Genedani S, Franco R, Agnati LF (2007) Adenosine receptor–dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 92:210-217.

Schiffrin SN, Fisone G, Moreasco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277-292.

Taura J, Valle-León M, Sahlihom K, Watanabe M, Van Craenenbroeck K, Fernández-Dueñas V, Ferré S, Ciruela F (2017) Behavioral control by striatal adenosine $A_2A_R$-dopamine $D_2_R$ receptor heteromers. Genes Brain Behav doi: 10.1111/gbb.12432.

Trilloeff P, Rives ML, Urizar E, Piskorowski RA, Vishwasrao HD, Carrillón J, Schmaus C, Slatmann M, Gullberg M, Javitch JA (2011) Detection of antigen interactions ex vivo by proximity ligation assay: endogenous dopamine $D_2$-adenosine $A_2A$ receptor complexes in the striatum. Biotechniques 51:111-118.

Vallano A, Fernandez-Duenas V, Pedros C, Arnau JM, Ciruela F (2011) An update on adenosine $A_2A$ receptors as drug target in Parkinson’s disease. CNS Neur Disord Drug Targets 10:659-669.