Adenosine A2A receptors and A2A receptor heteromers as key players in striatal function

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A very significant density of adenosine A2A receptors (A2ARs) is present in the striatum, where they are preferentially localized postsynaptically in striatopallidal medium spiny neurons (MSNs). In this localization A2ARs establish reciprocal antagonistic interactions with dopamine D2 receptors (D2Rs). In one type of interaction, A2AR and D2R are forming heteromers and, by means of an allosteric interaction, A2AR counteracts D2R-mediated inhibitory modulation of the effects of NMDA receptor stimulation in the striatopallidal neuron. This interaction is probably mostly responsible for the locomotor depressant and activating effects of A2AR agonist and antagonists, respectively. The second type of interaction involves A2AR and D2R that do not form heteromers and takes place at the level of adenylyl cyclase (AC). Due to a strong tonic effect of endogenous dopamine on striatal D2R, this interaction keeps A2AR from signaling through AC. However, under conditions of dopamine depletions or with blockade of D2R, A2AR-mediated AC activation is unleashed with an increased gene expression and activity of the striatopallidal neuron and with a consequent motor depression. This interaction is probably the main mechanism responsible for the locomotor depression induced by D2R antagonists. Finally, striatal A2ARs are also localized presynaptically, in cortico-striatal glutamatergic terminals that contact the striato-nigral MSN. These presynaptic A2AR heteromerize with A1 receptors (A1Rs) and their activation facilitates glutamate release. These three different types of A2ARs can be pharmacologically dissected by their ability to bind ligands with different affinity and can therefore provide selective targets for drug development in different basal ganglia disorders.

Keywords: adenosine A2A receptor, striatum, receptor heteromers, dopamine receptors, cannabinoid receptors

POSTSYNAPTIC STRIATAL ADENOSINE A2A RECEPTORS

A very significant density of adenosine A2A receptors (A2ARs) is present in the striatum (Rosin et al., 1998; Hettinger et al., 1998; Schiffmann et al., 2007; Quiroz et al., 2009), where they are preferentially localized postsynaptically in the soma and dendrites of GABAergic striatopallidal. These neurons also show a high density of dopamine D2 receptors (D2Rs) and there is clear evidence for the existence of postsynaptic mechanisms in the control of glutamatergic neurotransmission to the enkephalinergic medium spiny neuron (MSN) by at least two reciprocal antagonistic interactions between A2ARs and D2Rs (Ferré et al., 2008). In one type of interaction, stimulation of A2AR counteracts the D2R-mediated inhibitory modulation of NMDA receptor (NMDAR)-mediated effects, which include modulation of Ca2+ influx, transition to the up-state and neuronal firing (Azdad et al., 2009; Higley and Sabatini, 2010; Figure 1). This interaction seems to be mostly responsible for the locomotor depressant and activating effects of A2AR agonists and antagonists, respectively (Ferré et al., 2008; Orru et al., 2011), which correlates with the results of behavioral experiments showing that A2AR activation or blockade decreases or increases, respectively, the motor effects elicited by D2R activation (Ferré et al., 2008).

Initially, the main mechanism responsible for this A2AR–D2R interaction was attributed to what it was described as an “intramembrane interaction,” by which activation of A2AR could decrease the affinity of an adjacent D2R for agonists in striatal membrane preparations (Ferré et al., 1991). It was afterward hypothesized that this kind of intramembrane interaction was a biochemical property of receptor heteromers with important functional implications (Zoli et al., 1993). A receptor heteromer is now defined as a macromolecular complex composed of at least two (functional) receptor units with biochemical properties that are demonstrably different from those of its individual components (Ferré et al., 2009). The term “intramembrane interaction” is now known as “allosteric interaction in the receptor heteromer,” which is defined as an intermolecular interaction by which binding of a ligand to one of the receptor units in the receptor heteromer changes the binding properties of another receptor unit (Ferré et al., 2009). Another definition recently introduced in the field of receptor heteromers is “biochemical fingerprint,” which is a
biochemical characteristic of a receptor heteromer that can be used for its identification, even in a native tissue (Ferré et al., 2009). The introduction of this concept is important in view of the difficulty in demonstrating receptor heteromers in native tissues. Biophysical techniques, such as bioluminescence and fluorescence resonance energy transfer (BRET and FRET) techniques can be easily applied in artificial cell systems to demonstrate receptor heteromerization (Bouvier, 2001), but not in native tissues. Recent technological advances, using receptor labeling with selective fluorescent ligands, have allowed the demonstration of receptor homomers with time-resolved FRET in a native tissue (oxytocin receptor homomers in mammary glands; Albizu et al., 2010). However, this required the use of high quantities of a tissue with high expression of the receptor under study (Albizu et al., 2010).

The A2AR–D2R allosteric interaction, in fact, constitutes a biochemical fingerprint of the A2AR–D2R heteromer, since it depends on the proper quaternary structure of the heteromer. Thus, it has been recently shown that disruption of an electrostatic interaction between identified intracellular domains of the A2AR and D2R leads to a significant modification of the quaternary structure of the heteromer and to the disappearance of the A2AR–D2R allosteric interaction (Borroto-Escuela et al., 2010a; Navarro et al., 2010). The electrostatic interaction in the A2AR–D2R heteromer involves an arginine-rich epitope of the third intracellular loop (3IL) of the D2R and a phosphorylated residue localized in the C terminus of the A2AR (Woods and Ferré, 2005; Navarro et al., 2010). It is important to point out that this electrostatic interaction is not directly involved in the A2AR–D2R heteromer interface, which seems to be mostly determined by direct interactions between transmembrane domains (Borroto-Escuela et al., 2010b; Navarro et al., 2010).

A closer look to recent electrophysiological experiments (Azdad et al., 2009; Higley and Sabatini, 2010) indicates that, although useful as a biochemical fingerprint, the allosteric interaction in the receptor heteromer does not play a main role in the antagonistic A2AR–D2R-mediated functional interaction. In the study by Azdad et al. (2009), the D2R-mediated response consisted on the counteraction of NMDAR-mediated increase in firing rate by enkephalinergic MSNs (analyzed by patch-clamp experiments in identified striatal D2R-expressing MSNs). In this experimental setting, application of an A2AR agonist did not produce any significant effect on its own, but completely blocked the D2R-mediated response. Remarkably, this interaction was dependent on the integrity of the quaternary structure of the A2AR–D2R heteromer. Thus, the counteracting effect of the A2AR agonist disappeared after the application of peptides that selectively disrupted the intracellular electrostatic interaction (Azdad et al., 2009). Importantly, the counteracting effect of the A2AR agonist was detected in the presence of a high concentration of the D2R agonist, which should be able to surmount a decrease in the affinity of the D2R caused by A2AR occupation (Ferré et al., 1991). Therefore, although it might still be involved, the allosteric interaction, which leads to a lower affinity of D2R for dopamine when adenosine is activating A2AR, does not seem to be the main mechanism underlying the A2AR–D2R functional interaction in the A2AR–D2R heteromer.

The same intracellular arginine-rich epitope of the D2R that is involved in the electrostatic interaction with A2AR in the A2AR–D2R heteromer has been demonstrated to bind to calmodulin and also to be fundamental for the activation of Gβγ proteins (Bofill-Cardona et al., 2000; Navarro et al., 2009). Since calmodulin binding to the same epitope of the D2R impairs its ability to signal
through Gi/o proteins (Bofill-Cardona et al., 2000), it is likely that binding of the C terminus of the A2A R to the same epitope reduces the capacity of the D2 R to bind calmodulin and to signal through Gi/o proteins. In fact, it has recently been shown that the binding of calmodulin to the A2A R–D2 R heteromer is occurring within the proximal portion of the A2A R but not with the D2 R (Navarro et al., 2009). It is possible that agonist binding to the A2A R induces a conformational change in the A2A R–D2 R heteromer that causes an even further impairment in the coupling of the D2 R to the Gi/o protein. Thus, it seems that, in the A2A R–D2 R heteromer, D2 R does not signal through Gi/o proteins or that its main signaling is by a G-protein-independent mechanism. However, the recent study by Higley and Sabatini (2010) suggests that the D2 R-mediated inhibitory modulation of NMDAR-mediated Ca²⁺ signaling in the enkephalinergic MSN is mediated by PKA and, therefore, most probably related to the ability of D2 R to couple to Gi/o and to inhibit adenylyl cyclase (AC). Interestingly, in these experiments (and in agreement with the experiments by Azdad et al., 2009), an A2A R agonist did not produce any significant effect on its own, but counteracted the effect of a D2 R agonist. Thus, although Higley and Sabatini (2010) suggested that this interaction between A2A R and D2 R takes place at the AC level, it shows similar characteristics to the A2A R–D2 R heteromer-dependent interaction. In summary, A2A R–D2 R heteromers seem to play a key role in the modulation of NMDAR-mediated signaling in the enkephalinergic MSN, but the molecular mechanisms involved in these A2A R–D2 R–NMDAR interactions are yet to be determined.

In addition to the antagonistic A2A R–D2 R receptor interaction in the A2A R–D2 R heteromer, D2 R stimulation impedes A2A R to signal through AC (Kull et al., 1999; Chen et al., 2001; Hillion et al., 2002; Håkansson et al., 2006; Figure 1). This D2 R–A2A R interaction takes place at the second messenger level, and stimulation of Gi/o-coupled D2 R counteracts the effects of Gαolf-coupled A2A R (Ferré et al., 2007, 2008). Due to a strong tonic effect of endogenous dopamine on striatal D2 R, this interaction keeps A2A R from signaling through AC. However, under conditions of dopamine depletion or with pharmacological D2 R blockade, A2A R-mediated signaling through the cAMP–PKA cascade may be unleashed. Antagonism of D2 R is biochemically associated with a significant increase in the phosphorylation of PKA-dependent substrates, which increases gene expression and the activity of the enkephalinergic MSN, producing locomotor depression (reviewed in Ferré et al., 2008). This appears to be the main mechanism responsible for the locomotor depression induced by D2 R antagonists. Thus the motor depressant and most biochemical effects induced by pharmacologic blockade of D2 R may be counteracted by pharmacological blockade of A2A R (Chen et al., 2001; Håkansson et al., 2006).

The two reciprocal antagonistic interactions, A2A R toward D2 R (A2A R–D2 R) and D2 R toward A2A R (D2 R–A2A R), take place simultaneously in the same cell, which suggest that are most likely mediated by the existence of at least two different populations of postsynaptic striatal A2A R in the enkephalinergic MSN (Ferré et al., 2008). One population would be forming heteromers with D2 R and would determine that A2A R stimulation inhibits D2 R-mediated signaling (A2A R–D2 R interaction), while another population would not be forming heteromers with D2 R and would determine that D2 R stimulation inhibits A2A R-mediated signaling (D2 R–A2A R interaction). This second population of postsynaptic A2A R would either not form heteromers or would form heteromers with other receptors, such as glutamate mGluR receptors (mGluRs; Ferré et al., 2002; Figure 1). Importantly, heteromerization of A2A R with mGluR is associated with a synergistic effect upon A2A R and mGluR co-activation at the level of AC and MAPK, providing a physiological mechanism by which A2A R can overcome the D2 R–A2A R interaction (Ferré et al., 2002; Nishi et al., 2003). Co-stimulation of A2A R and mGluR in vivo, with the central administration of selective agonists, allowed A2A R to get rid of the inhibitory effect of the D2 R and signal through the cAMP–PKA cascade (Ferré et al., 2002). Since this A2A R–D2 R–mGluR interaction could be demonstrated in animal models of Parkinson's disease (Popoli et al., 2001; Kachroo et al., 2005), it was postulated that co-administration of A2A R and mGluR antagonists could be useful as a therapeutic strategy in this disease (Popoli et al., 2001).

Still a third population of postsynaptic A2A R would form heteromers with cannabinoid CB1 receptors (CB1 Rs; Carriba et al., 2007; Figure 1). In this heteromer, activation of A2A R is necessary to allow CB1 R-mediated signaling. Thus, in a human neuroblastoma cell line, CB1 R-mediated inhibition of AC activity was found to be completely dependent on A2A R co-activation (Carriba et al., 2007). Similarly, several biochemical effects of CB1 R agonists in primary striatal cell cultures and striatal slices have been shown to depend on A2A R co-activation (Yao et al., 2003; Andersson et al., 2005). Accordingly, Tébano et al. (2009) reported that the depression of synaptic transmission induced by a CB1 R agonist in cortico-striatal slices was prevented by A2A R antagonists and also by the conditional genetic blockade of striatal postsynaptic A2A R. The permissive effect of A2A R toward CB1 R function did not seem to occur presynaptically, as the ability of the CB1 R agonist to increase the R2/R1 ratio under a protocol of paired-pulse stimulation was not modified by an A2A R antagonist (Tébano et al., 2009). These results would predict that A2A R antagonists should produce similar behavioral effects than CB1 R antagonists and, in fact, pharmacological or genetic inactivation of A2A R reduces the motor depressant, cataleptic, and rewarding effects of CB1 R agonists (Soria et al., 2004; Andersson et al., 2005; Carriba et al., 2007; Justinova et al., 2011). Significantly, it has been recently reported that low doses of an A2A R antagonist (MSX-3) reduce in squirrel monkeys self-administration of THC and anandamide, but not cocaine (Justinova et al., 2011).

Although the studies just mentioned indicate that the motor (depressant) effects of CB1 R agonists might depend on adenosine A2A R receptor signaling, a recent study by Lerner et al. (2010) suggested quite the opposite, that CB1 R signaling mediates the locomotor-activating effects of A2A R antagonists. Thus, pharmacological or genetic inactivation of CB1 R reduced the locomotor activation induced by an A2A R antagonist in mice habituated to the testing environment (Lerner et al., 2010). The mechanistic explanation of this interaction is related to the previously reported D2 R agonist-mediated endocannabinoid release by the enkephalinergic MSN, which by retrograde signaling would inhibit glutamate release by stimulating CB1 R localized in glutamatergic terminals. This would lead to a decreased stimulation of the striatopallidal MSN, which would produce locomotor activation (Kreitzer and
Malenka, 2007). In fact, Kreitzer and Malenka (2007) advocated that, instead of direct postsynaptic effects, such as the previously mentioned D2R-mediated modulation of NMDAR-mediated signaling (Azad et al., 2009; Higley and Sabatini, 2010), this indirect and endocannabinoid-mediated presynaptic effect is the main mechanism by which D2R stimulation produces inhibition of the enkephalinergic MSN function. According to Lerner et al. (2010), an A2AR antagonist would then produce locomotor activation by disinhibiting a tonic A2AR-mediated inhibition of D2R-mediated endocannabinoid release. However, this hypothesis would predict that CB1R agonists and antagonists should produce locomotor activation and depression, respectively, and that CB1R blockade should counteract the motor effects of D2 receptor agonists. This is the opposite of what has been reported in previous studies (for a recent review, see Ferré et al., 2010). To reevaluate the findings by Lerner et al. (2010) we studied in detail the effects of pharmacological interactions between A2AR antagonists and CB1R antagonists on the locomotor activity in rats not habituated to the testing environment (Orru et al., submitted). Whereas we could indeed reproduce the results by Lerner et al. (2010) showing that a CB1R antagonist significantly decreases the locomotor effects induced by an A2AR antagonist, we found that the CB1R antagonist also produces a comparable decrease in locomotion in vehicle-treated animals (statistical analysis indicated that the locomotor effects of A2AR and CB1R antagonists were not interrelated). It was therefore the use of habituated animals (which display very low locomotor activity in the testing environment) that masked the depressive effect of CB1R antagonist in the vehicle-treated animals in the study by Lerner et al. (2010).

In addition to the three populations of postsynaptic striatal A2AR so far reported, there is also experimental evidence for a potentially more complex picture, which includes the possibility of receptor heteromers. Thus, using a new biophysical-based technology, sequential resonance energy transfer (SRET), and bimolecular fluorescence complementation plus BRET, evidence for A2AR–CB1R–D2AR and A2AR–D2R–mGlu5R heteromers in transfected cells has been recently obtained (Carriba et al., 2008; Cabello et al., 2009; Navarro et al., 2010). Mutation experiments indicated that the interactions of the intracellular domains of the CB1R receptor with A2AR and D2R are fundamental for the correct formation of the quaternary structure needed for the function (MAPK signaling) of the A2AR–CB1R–D2AR heteromers. It should be noted that the analysis of MAPK signaling in striatal slices of CB1R KO mice and wild-type littermates supports the existence of A2AR–CB1R–D2R receptor heteromers in the brain (Navarro et al., 2010). Despite the stoichiometry of the different populations of postsynaptic striatal A2AR heteromers (and homomers) is not known, taking into account the very high density of A2ARs and D2Rs in the enkephalinergic MSN, we postulate that A2AR and D2R homomers and A2AR–D2R heteromers are the most common receptor populations, followed by combinations of those populations with CB1R and mGlu5R.

It is also of importance to mention that there is also evidence for the existence of A2AR receptors, also co-localized with D2Rs, in the somatodendritic and nerve terminal regions of the cholinergic striatal interneurons and that their interactions modulate acetylcholine release (James and Richardson, 1993; Jin et al., 1993; Preston et al., 2000; Tozzi et al., 2011). The study by Jin et al. (1993) showed evidence for an antagonistic A2AR–D2AR interaction in the modulation of striatal acetylcholine release. Thus, A2AR stimulation counteracted the ability of D2R activation to inhibit acetylcholine release. Similarly, a recent study showed that A2AR blockade potentiates D2R-mediated modulation of acetylcholine release (Tozzi et al., 2011), again indicating the existence of an antagonistic A2AR–D2R interaction and, probably, A2AR–A2AR heteromers in striatal cholinergic interneurons.

**PRESYNAPTIC STRIATAL ADENOSINE A2A RECEPTORS**

Striatal A2ARs are not only localized postsynaptically but also presynaptically, in glutamatergic terminals, where they heteromerize with A1 receptors (A1Rs) and where they perform a fine-tuned modulation of glutamate release (Ciruela et al., 2006; Quiroz et al., 2009; Figure 1). Thus, A1R–A2AR heteromers seem to work as a concentration-dependent switch (Ferré et al., 2007), with adenosine acting primarily at A1Rs at low concentrations, and at both A1R and A2ARs at higher concentrations. Activation of the A1R in the A1R–A2AR heteromer produces inhibition of glutamate release, while the additional activation of the A2AR produces the opposite effect, on a mechanism that seems to involve an allosteric modulation in the receptor heteromer and interactions at the G protein level (Ciruela et al., 2006; Ferré et al., 2007). Interestingly, presynaptic A2ARs are preferentially localized in glutamatergic terminals of cortico-striatal afferents to the dynorphinergic MSN (Quiroz et al., 2009). Apart from morphological evidence provided by immunohistochemical and electron microscopy experiments, patch-clamp experiments in identified enkephalinergic and dynorphinergic MSNs provided a functional demonstration of the segregation of striatal presynaptic A2ARs. Thus, an A2AR agonist and an A2AR receptor antagonist significantly increased and decreased, respectively, the amplitude of excitatory postsynaptic currents induced by the intrastriatal stimulation of glutamatergic afferents measured in identified enkephalinergic, but not dynorphinergic MSNs. Mean-variance analysis indicated a presynaptic locus for the A2AR-mediated modulation (Quiroz et al., 2009). Thus, there seems to be a selective A2AR-mediated modulation of glutamate release to the dynorphinergic MSN, which is in disagreement with the recently proposed role of postsynaptic A2ARs in the modulation of glutamate release to the enkephalinergic MSN (Lerner et al., 2010).

The powerful modulatory role of presynaptic A2ARs on striatal glutamate release was first demonstrated with in vivo microdialysis experiments by Popoli et al. (1995), who showed that the striatal perfusion of an A2AR agonist produced a very pronounced increase in the basal striatal extracellular concentrations of glutamate. Also intrastriatal perfusion of an A2AR antagonist through a microdialysis probe could significantly counteract striatal glutamate release induced by cortical electrical stimulation in the orofacial premotor cortex (Quiroz et al., 2009). A striking unexpected finding was that the counteraction of glutamate release was also accompanied by a complete counteraction of the jaw movements induced by the cortical electrical stimulation, demonstrating the very important role of presynaptic A2ARs in the control of cortico-striatal glutamatergic neurotransmission. By combining cortical electrical stimulation and recording of EMG activity of the mastication...
As previously suggested (Ferré et al., 2007), activation of presynaptic A2AR agonist-mediated stimulation of glutamate release (an effect postulated (Ferré et al., 2010; Justinova et al., 2011). However, this hypothesis, at least in those terminals establishing contact with the dynorphinergic MSNs under basal conditions. The results by Shen et al. (2008) about the differential effects of A2AR antagonists on psychostimulant-induced locomotor activation in WT versus conditional striatal postsynaptic A2AR KO mice (potentiation versus counteraction, respectively) support this hypothesis.

As previously suggested (Ferré et al., 2007), activation of presynaptic A2ARs seems to be highly dependent on the level of adenosine generated upon cortico-striatal glutamatergic input. Striatal D2Rs are also localized presynaptically, in dopaminergic and glutamatergic terminals (Higley and Sabatini, 2010), giving the frame for the existence of interactions with A2ARs at least in those terminals establishing contact with the dynorphinergic MSN. The experimental evidence suggest that there is also a presynaptic D2R–A2AR interaction by which D2R activation tonically inhibits the ability of endogenous adenosine to produce an A2AR-mediated increase in the basal extracellular levels of glutamate. Thus, dopamine denervation significantly potentiates A2AR agonist-mediated stimulation of glutamate release (Tanganelli et al., 2004). This has the biochemical characteristics of an interaction between A2ARs and D2Rs at the AC level and not forming A2AR–D2R heteromers. Furthermore, results Rodrigues et al. (2005) have also demonstrated the existence of mGlu5Rs in striatal glutamatergic terminals co-localized with A2ARs and which facilitate glutamate release in a synergistic manner. The interplay between adenosine- and dopamine-mediated actions at the presynaptic level is therefore affected by the occurrence of mGlu5Rs.

The presynaptic localization of CB1Rs in striatal glutamatergic terminals is well established, and therefore they can be co-localized with A2AR in terminals establishing contact with the dynorphinergic MSN (Ferré et al., 2010). The existence of A2AR–CB1R heteromers in striatal glutamatergic terminals which could mediate the reinforcing effects of cannabinoids has been recently postulated (Ferré et al., 2010; Justinova et al., 2011). However, a recent study by Martire et al. (2011) indicates that cannabinoid/adenosine functional interactions result from an interaction at the second messenger level. In the frame of heteromerization A2AR activation should facilitate the Gq/11-mediated effect of CB1R activation measured, as inhibition of glutamate release. Nevertheless, Martire et al. (2011), by studying extracellular field potentials recordings in cortico-striatal slices and superfused striatal nerve terminals, very convincingly showed that, instead, A2AR activation prevents CB1R-mediated inhibition of glutamate release. These results indicate that regulation of glutamate release by cannabinoids is not dependent on presynaptic A2AR–CB1R heteromers.

In summary, a great amount of available data indicates that, presynaptically, A2ARs form heteromers mostly with A1Rs. In addition, there seems to be a population of A2ARs not forming heteromers but establishing antagonistic interactions with D2Rs and CB1Rs and synergistic interactions with mGlu5Rs. Apart from co-expression, at this moment we do not know the variables that determine the ability of A2ARs to bind to different receptors to form different pre and postsynaptic heteromers. Thus, D2Rs are also localized presynaptically, but yet they do not seem to form heteromers with A2ARs. A2ARs could bind with more affinity to A1Rs than to D2Rs or particular scaffolding proteins could favor a particular A2AR heteromer. All these questions still need to be answered.

**TARGETING STRIATAL PRE AND POSTSYNAPTIC A2A RECEPTORS**

A surprising yet fundamental finding of a recent study is that several A2AR antagonists previously thought as being pharmacologically similar present different striatal pre and postsynaptic profiles (Orru et al., 2011). Six compounds already known as selective A2AR antagonists were first screened for their ability to block striatal pre and postsynaptic A2ARs with in vivo models. Locomotor activation was used to evaluate postsynaptic activity while PCC counteraction was used to determine presynaptic activity (see above). SCH-442416 and KW-6002, showed preferential pre and postsynaptic profiles, respectively, and four compounds, MSX-3, SCH-420814, SCHR-58261, and ZM-241385, showed mixed pre–postsynaptic profiles. Combining in vivo microdialysis with cortical electrical stimulation was used as an additional in vivo evaluation of presynaptic activity of A2A antagonists. In agreement with its preferential presynaptic profile, SCH-442416 significantly counteracted striatal glutamate release induced by cortical stimulation at a dose that strongly counteracted PCC but did not induce locomotor activation. On the other hand, according to its preferential postsynaptic profile, KW-6002 did not modify striatal glutamate release induced by cortical stimulation at a dose that produced a pronounced locomotor activation but did not counteract PCC.

Another important finding of the study by Orru et al. (2011) was that at least part of these pharmacological differences between A2AR antagonists could be explained by the ability of pre and postsynaptic A2AR to form different receptor heteromers, with A1R and D2R, respectively (see above). Radioligand-binding experiments were performed in cells stably expressing A2AR, A2AR–D2R heteromers, or A1R–A2AR heteromers to determine possible differences in the affinity of these different A2AR antagonists. Co-expression with A1R did not significantly modify the affinity of A2ARs for the different ligands, but co-expression with D2Rs decreased the affinity of all compounds, with the exception of...
The decrease in affinity upon co-expression with D2Rs was much less pronounced for ZM-241385, SCH-58261, MSX2, or SCH-420814, for which the affinity was reduced from two to about ninefold (Orru et al., 2011). Taking into account that these A2AR antagonists behaved qualitatively similar to the A2AR agonist CGS-21680 in terms of binding to A1R–A2AR and A2AR–D2R heteromers, it was expected that these four compounds compete equally for the binding of the endogenous agonist at presynaptic and postsynaptic sites. This would fit with the in vivo data, which showed that these compounds have a non-preferred pre- and postsynaptic profile. Yet, KW-6002 was the only antagonist whose affinity was not significantly different in cells expressing A2AR, A1R–A2AR heteromers, or A2AR–D2R heteromers. Thus, KW-6002 showed the best relative affinity for A2AR–D2R heteromers of all compounds, which can at least partially explain its preferential postsynaptic profile. Experiments performed with the non-selective adenosine receptor antagonist caffeine also showed a correlation between the in vivo data and the in vitro preference for postsynaptic A2AR-containing heteromers. It was previously reported that in transfected mammalian cells the affinity of A2AR for the non-selective adenosine receptor antagonist caffeine did not change when co-transfected with D2R, but it was significantly decreased (about 10 times) when co-transfected with A1R (Ciruela et al., 2006). As predicted, caffeine did not significantly reduce PCC at doses that produce pronounced motor activation (Zanoveli et al., in preparation).

It must be pointed out that to say that SCH-442416 is a selective presynaptic A2AR antagonist is an oversimplification. In fact, the in vitro data indicated that SCH-442416 binds equally well to the A2AR not forming heteromers than to the A2AR in the A1R–A2AR heteromer. Therefore, according to the previous description of the different populations of striatal A2ARs, SCH-442416 should also be effective at counteracting D2R antagonist-induced motor depression. In fact, at doses that are not producing locomotor activation (but that reduce PCC), SCH-442416 significantly counteracts the locomotor depression induced by the D2R antagonist raclopride (Orru et al., submitted). On the other hand, KW-6002 produced the same locomotor activation with or without co-administration with raclopride, in agreement with its ability to block the three populations of A2AR studied so far in vitro, A2AR, A2A–D2R, and A1R–A2AR. Importantly, KW-6002 also produced the same locomotor activation when co-administered with the A2AR agonist CGS-21680, while SCH-442416, at the same dose that counteracted the depressant effect of raclopride, did not significantly counteract the depressant effect of CGS-21680. These results, therefore agree with the hypothesis that the subpopulation of postsynaptic A2AR forming heteromers with D2R are mainly responsible for both the locomotor activation and depression induced by A2AR antagonists and agonists, respectively. In summary, SCH-442416 can be considered as a compound that at relatively low doses not only binds preferentially to presynaptic A2ARs localized in cortico-striatal glutamatergic terminals (Orru et al., 2011), but also to a subpopulation of postsynaptic A2ARs most probably not forming heteromers with D2Rs, but which function is tonically inhibited by D2Rs activated by endogenous dopamine. Interestingly, [11C]SCH-442416 has been used in rats, monkeys, and humans as a PET radioligand and shown to nicely label striatal A2ARs (Moresco et al., 2005; Schiffmann et al., 2007; Brooks et al., 2010). The low doses used in PET experiments indicate that [11C]SCH-442416 is mostly labeling presynaptic A2ARs and postsynaptic A2ARs that do not form heteromers with D2Rs. The use of [11C]SCH-442416 and other less selective radioligand in combination with cold SCH-442416 could allow the identification of the different populations of A2ARs in the human brain. The picture is still incomplete, and a further evaluation of the affinity of A2AR antagonists for A2AR–mGlu5R and A2AR–CB1 heteromers (and of heterotrimers) is needed. Nevertheless, the information so far available is very valuable to attempt the design of more efficient A2AR antagonists to be used in basal ganglia disorders.

**A2A RECEPTOR HETEROMERS AS TARGETS FOR DRUG DEVELOPMENT**

The results of the above mentioned studies support the notion that receptor heteromers may be used as selective targets for drug development. Main reasons are the very specific neuronal localization of receptor heteromers (even more specific than receptor subtypes themselves), and a differential ligand affinity of a receptor depending on its partner (or partners) in the receptor heteromer. Striatal A2AR-containing heteromers become particularly interesting targets, eventually useful for a variety of neuropsychiatric disorders. Blocking postsynaptic A2ARs in the enkephalinergic MSN should be beneficial for Parkinson’s disease because it would decrease the activity of the indirect striatal efferent pathway. On the one hand, one benefit would come from potentiating the effect of l-dopa or other dopamine receptor agonists on the D2R-mediated signaling in the A2AR–D2R heteromer. On the other hand, blockade of A2ARs not forming heteromers with D2Rs (but antagonistically interacting with D2R at the AC level) should counteract the effects of the disinhibited A2AR signaling. However, blocking presynaptic A2AR in glutamatergic terminals contacting dynorphinergic MSN (either forming or not heteromers with A1R) should decrease glutamatergic transmission through the direct striatal efferent pathway, thus decreasing motor activity and, therefore, decreasing the antiparkinsonian efficacy of A2AR antagonists. The most convenient A2AR antagonist to treat Parkinson’s disease patients would have more affinity for postsynaptic than for presynaptic receptors. Additionally, a selective blockade of presynaptic A2ARs should be useful in dyskinetic disorders such as Huntington’s disease and could also be useful in obsessive–compulsive disorders and drug addiction. Effective treatment of l-dopa-induced dyskinesia using “presynaptic” A2AR antagonists would be a possibility to explore.
The results by Orru et al. (2011) give a mechanistic explanation to the already reported antiparkinsonian activity of KW-6002 and suggest that SCH-442416 could be useful for the treatment of dyskinetic disorders, obsessive–compulsive disorders and in drug addiction. Medicinal chemistry and in silico modeling should help in elucidating the molecular properties that determine the particular pharmacological profile of SCH-442416 and KW-6002, which may be used as lead compounds to obtain, respectively, more effective antidyskinetic and antiparkinsonian compounds.

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