An Update on Adenosine A_{2A}-Dopamine D_2 Receptor Interactions: Implications for the Function of G Protein-Coupled Receptors

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Abstract: Adenosine A_{2A}-dopamine D_2 receptor interactions play a very important role in striatal function. A_{2A}-D_2 receptor interactions provide an example of the capabilities of information processing by just two different G protein-coupled receptors. Thus, there is evidence for the coexistence of two reciprocal antagonistic interactions between A_{2A} and D_2 receptors in the same neurons, the GABAergic enkephalinergic neurons. An antagonistic A_{2A}-D_2 intramembrane receptor interaction, which depends on A_{2A}-D_2 receptor heteromerization and $G_{q/11}$-PLC signaling, modulates neuronal excitability and neurotransmitter release. On the other hand, an antagonistic A_{2A}-D_2 receptor interaction at the adenylyl-cyclase level, which depends on $G_{s/olf}$- and $G_{i/o}$- type V adenylyl-cyclase signaling, modulates protein phosphorylation and gene expression. Finally, under conditions of upregulation of an activator of G protein signaling (AGS3), such as during chronic treatment with addictive drugs, a synergistic A_{2A}-D_2 receptor interaction can also be demonstrated. AGS3 facilitates a synergistic interaction between $G_{s/olf}$ - and $G_{i/o}$-coupled receptors on the activation of types II/IV adenylyl cyclase, leading to a paradoxical increase in protein phosphorylation and gene expression upon co-activation of A_{2A} and D_2 receptors. The analysis of A_2-D_2 receptor interactions will have implications for the pathophysiology and treatment of basal ganglia disorders and drug addiction.

Key Words: Adenosine A_{2A} Receptor, Dopamine D_2 Receptor, G Protein-Coupled Receptors, Receptor Heteromers, Striatum, Basal Ganglia Disorders, Drug Addiction.

LOCALIZATION OF THE A_{2A}-D_2 RECEPTOR HETEROMER

Applying a broad definition of "neurotransmitter" [1], adenosine can be considered as an important neurotransmitter in the CNS, which acts through different subtypes of G protein-coupled receptors (GPCRs). From the four cloned adenosine receptors (adenosine A_1, A_{2A}, A_{2B} and A_3 receptors), A_1 and A_{2A} receptors are the main targets for the physiological effects of adenosine in the brain [2]. A_1 receptor is widely distributed in the brain, including the striatum, while A_{2A} receptor is mostly concentrated in the striatum [2,3]. It is becoming increasingly obvious that the modulatory role of adenosine in the striatum is related to the ability of A_1 and A_{2A} receptors to heteromerize with themselves and with other GPCRs, such as dopamine, glutamate, cannabinoid and ATP receptors [4-14]. The present review focuses on the role of one particular adenosine receptor heteromer, the one constituted by the A_{2A} and the dopamine D_2 receptor, which is already having important implications for the treatment of neuropathologies involving the striatum (see below).

Striatal medium spiny neurons are GABAergic efferent neurons which constitute more that 95% of the striatal neuronal population. They receive two main afferents, cortical-limbic-thalamic glutamatergic inputs and dopaminergic mesencephalic inputs, from the substantia nigra pars compacta and the VTA. These inputs converge in the dendritic spine, with the glutamatergic input making synaptic contact with the head of the dendritic spine and the dopaminergic input making synaptic contact with the neck of the dendritic spine [15,16]. The dendritic spine, the glutamatergic terminal, the dopaminergic terminal and astroglial processes that wrap the glutamatergic synapse constitute the most common local module in the striatum, which we have recently called striatal spine module [16]. In the striatal spine module adenosine plays a very important role in the modulation of both glutamatergic and dopaminergic neurotransmission [14,16,17].

It was initially thought that most extracellular adenosine came from intracellular adenosine as a product of ATP, due to an increased metabolic demand of the cell [17]. However, recent studies suggest that astroglia plays a very important role in the production of extracellular adenosine. Astrocytes express glutamate and ATP receptors, which when activated induce astrocytes to release glutamate and ATP, which can then be converted to adenosine by means of ectonucleotidases [18-20]. This adds more relevance to the already known key role of astrocytes in the computation of information in the striatal spine module [16,18,19]. Finally, increasing evidence suggests that ATP is co-released with glutamate by the glutamatergic terminals and converted to adenosine by ectonucleotidases [14,16].

There are two subtypes of GABAergic striatal efferent neurons, the GABAergic striopallidal neuron, which can be called GABAergic enkephalinergic neuron, since it expresses the peptide enkephalin, and the GABAergic striatonigral-striatoentopeduncular neuron, which can be called GABAergic dynorphynergic neuron, since it expresses the peptide dynorphin (and also substance P). The GABAergic enkephalinergic neuron predominantly expresses dopamine and adenosine receptors of the D_2 and A_{2A} receptor subtype [3,9,14-17,21-23], while the GABAergic dynophynergic neuron expresses dopamine and adenosine receptors of the D_1 and A_1 subtype [3,9,23].

We found evidence for the existence of A_{2A}-D_2 receptor interactions that modulate the function of the GABAergic enkephalinergic neuron and A_1-D_1 receptor interactions that modulate the function of GABAergic dynorphinergic neuron [23]. We and other authors also found evidence for the existence of selective heteromerization of A_{2A} and D_2 receptors and A_1 and D_1 receptors in transfected cells [4,8,10,11] and found biochemical characteristics of these receptor heteromers, called "intramembrane receptor-receptor interactions" [9], which could also be identified in the striatum (reviewed in ref.

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22), therefore demonstrating the existence of A_{2A}-D_2 and A_1-D_1 receptor heteromers in the brain [14,24,25].

Then, going back to the striatal spine module, we have to differentiate between two types of modules, the one centered at the dendritic spine of the GABAergic enkephalinergic neuron, which contains A_{2A}-D_2 receptor heteromers, and the one centered at the dendritic spine of the dynorphinergic neuron, which contains A_1-D_1 receptor heteromers. In addition to the A_{2A}-D_2 and A_1-D_1 receptor heteromers, we have identified A_1-A_{2A} receptor heteromers in the glutamatergic terminals of, most probably, both striatal spine modules [12]. Furthermore, there is functional evidence for the existence of presynaptic interactions between A_{2A} and D_2 (or maybe D_4) receptors that modulate striatal glutamate release (see below).

THE ANTAGONISTIC A_{2A}-D_2 INTRAMEMBRANE RECEPTOR INTERACTION

A_{2A}-D_2 receptor heteromerization was first demonstrated in mammalian transfected cells with co-immunoprecipitation, and fluorescence and bioluminescence resonance energy transfer techniques (FRET and BRET, respectively) [10,11] (Fig. **1**). By using computerized modeling, pull-down and mass spectrometry techniques, it was shown that this heteromerization depends on an electrostatic interaction between an arginine-rich epitope of the N-terminal segment of the third intracellular loop (NI3L) of the D_2 receptor and a phosphate group in the C-terminus of the A_{2A} receptor [10,26,27]. FRET and BRET, however, are difficult techniques to implement in tissues and the demonstration of the A_{2A}-D_2 receptor heteromer was demonstrated by indirect means, by identifying a biochemical characteristic, what we have called a "biochemical fingerprint" of the heteromer [14,24,25].

The concept of "intramembrane receptor interactions" was first described by Luigi Agnati and Kjell Fuxe more than 20 years ago (reviewed in ref. 9). In these interactions, stimulation of one receptor changes the binding characteristics of an adjacent receptor in membrane preparations from brain tissue or transfected cells. It is now recognized that intramembrane receptor interactions constitute a common biochemical characteristic of receptor heteromers [14,23-25]. The antagonistic A_{2A}-D_2 intramembrane receptor interaction has been repeatedly reported by different research groups in membrane preparations from different transfected cell lines and from human and rat striatum [28-34]. In these membrane preparations, the addition of a selective A_{2A} receptor agonist decreases the ability of dopamine (or a D_2 receptor agonist) to displace the binding of a selective D_2 receptor radioligand [28-34] (Fig. **2a**).

The antagonistic A_{2A}-D_2 intramembrane receptor interaction seems to determine the ability of A_{2A} receptors to control the inhibitory role of D_2 receptors on neuronal excitability and neurotransmitter release in the GABAergic enkephalinergic neuron. It was demonstrated by means of *in vivo* microdialysis experiments that the perfusion of a D_2 receptor agonist in the dorsal striatum, localization of the cell bodies of the GABAergic enkephalinergic neuron, leads to a decrease in the extracellular levels of GABA in the ipsilateral globus pallidus, localization of the nerve terminals of the enkephalinergic neurons [35]. On the other hand, the striatal perfusion of an A_{2A} receptor agonist did not produce any significant effect, but it completely counteracted the effect of the D_2 receptor agonist [35]. Thus, in this experimental setting, an A_{2A} receptor agonist behaves as a D_2 receptor antagonist. In the nucleus accumbens (ventral striatum), there is a more tonic effect of D_2 receptor stimulation by endogenous dopamine on neurotransmitter release by the GABAergic enkephalinergic neuron [36]. Also in this experimental setting the striatal perfusion of an A_{2A} receptor agonist produced the same effects than a D_2 receptor antagonist, i.e., an increase in the extracellular levels of GABA in the ipsilateral ventral pallidum [36].

The striatal output is determined by the bursting activity of the GABAergic striatal efferent neurons. These bursts are driven by cortico-striatal inputs that depolarize the GABAergic enkephalinergic and dynorphinergic neurons from their resting hyperpolarized membrane potential around -80 mV, the down-state, to a more depolarized level near -55 mV, the up-state [37]. These down- to up-state transitions require channels that can be regulated by striatal transmitters acting through GPCRs, such as the interacting A_{2A} and D_2 receptors in the GABAergic enkephalinergic neurons. In a re-

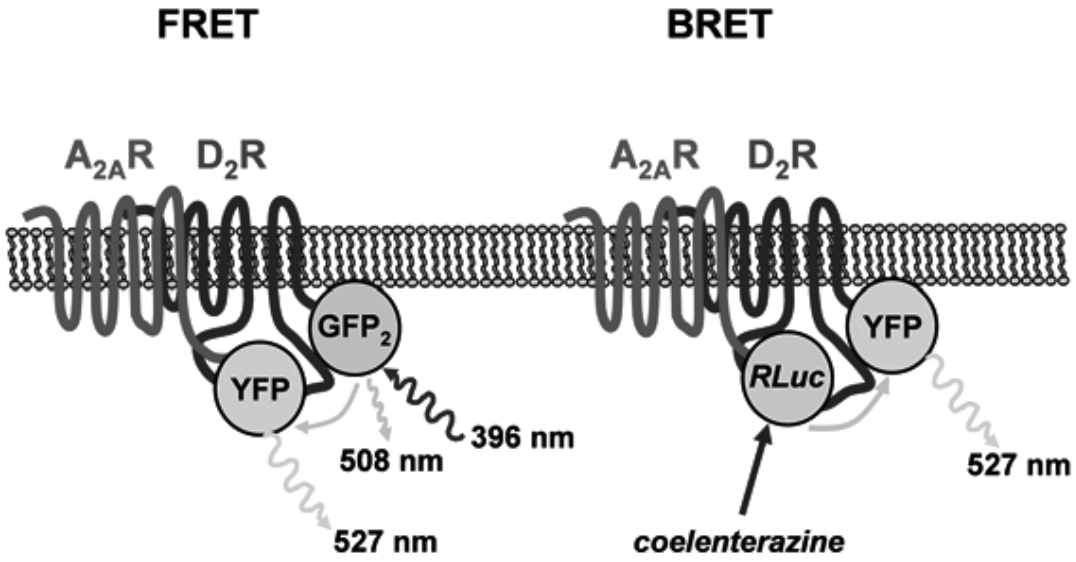

Fig. (1). Bioluminiscence and fluorescence resonance energy transfer (BRET and FRET, respectively) techniques allow the demonstration of neurotransmitter receptor heteromers in the natural environment of the living cell. FRET can be obtained when two fluorescent proteins, one acting as a donor and the other acting as acceptor, are close enough (within 10 nm). Therefore, to demonstrate receptor dimerization, cDNA constructs of one receptor fused to the fluorescent donor and another receptor fused to the fluorescent acceptor are prepared and transfected in a heterologous cell system. Different versions of the green fluorescent protein (GFP) are currently used as donors whereas yellow fluorescent proteins (YFPs) are used as acceptors. If the two receptors are forming dimers, the acceptor fluorescent signal is detected after donor excitation. In BRET, instead of a fluorescent donor one of the receptors is fused to the luminescent protein, *Renilla luciferase* (*Rluc*), which upon addition of a substrate (coelenterazine or Deep Blue C) allows energy transfer and excitation of the fluorescent acceptor.

cent study, perforated patch clamp recordings on acute brain slices, together with the loading of competitive peptides to block specific protein-protein interactions, were used to characterize the role of A_{2A}-D_2 receptors interactions in the modulation of down- to up-state transitions, modeled *in vitro* by the application of NMDA. A D_2 receptor agonist abolished the firing in up-state and inhibited the down/up-state transition in the GABAergic enkephalinergic neurons by a mechanism involving the regulation of L-type calcium channel $Ca_V1.3$ through protein-protein interactions with scaffold proteins Shank1/3 (Azdad *et al*., Society for Neuroscience Abstracts, 2007). On the other hand, the A_{2A} receptor agonist CGS 21680 did not induce any modification in state transition or in the firing frequency, but it totally reversed the effects of D_2 receptor activation (Azdad *et al*.). This action was blocked by the selective A_{2A} receptor antagonist SCH 58261 (1μM) and was absent in A_{2A} receptor knock-out mice (Azdad *et al*.). The application of peptides containing the same aminoacid sequence than the epitopes involved in A_{2A}-D_2 receptor heteromerization counteracted the ability of A_{2A} receptor activation to antagonize the effect of D_2 receptor activation (Azdad *et al*.). This demonstrates that A_{2A}-D_2 receptors heteromerization is strictly mandatory for the A_{2A} receptor-mediated control of D_2 receptor-mediated modulation of the excitability of GABAergic enkephalinergic neurons.

These effects on neurotransmitter release and neuronal excitability are paralleled by effects on motor activity and other behavioral responses, where selective A_{2A} receptor agonists or antagonists respectively counteract or potentiate the motor activation induced by dopamine D_2 receptor agonists [38-42]. Consequently, we predicted 15 years ago that A_{2A} receptor antagonists could be useful in Parkinson's disease, especially potentiating the effects of L-dopa or D_2 receptor agonists [43]. In fact, in different experimental models of Parkinson's disease, A_{2A} receptor antagonists potentiate the motor activating effects of L-DOPA or D_2 receptor agonists (for review see ref. 42). Also in agreement, in the rodent dopamine-denervated striatum, local application of a D_2 receptor agonist potently inhibits the increased neuronal activity (compared with the non-denervated striatum) and this effect is counteracted or potentiated with application of A_{2A} agonists or antagonists, respectively [41]. Importantly, the A_{2A} receptor ligands did not have any significant effects on their own [41]. On the other hand, in patients with Parkinson's disease the association of L-DOPA and an A_{2A} receptor antagonist has already given promising therapeutic results (reviewed in ref. 44).

THE ANTAGONISTIC A_{2A}-D_2 RECEPTOR INTERACTION AT THE SECOND MESSENGER LEVEL

A_{2A} receptor, through its coupling to G_{olf} proteins, can potentially stimulate adenylyl-cyclase and activate the cAMP-PKA signaling pathway, with phosphorylation of several PKA substrates, such as DARPP-32, CREB and AMPA receptors and the consequent increase in the expression of different genes, such as *c-fos* or *preproenkephalin* in the GABAergic enkephalinergic neuron [3,9,14,16,23,24]. For instance, in CHO cells stably transfected with A_{2A} receptors, the addition of an A_{2A} receptor agonist produced cAMP accumulation, CREB phosphorylation and increase in *c-fos* expression [31]. In the same cell line we could demonstrate the existence of an antagonistic A_{2A}-D_2 intramembrane receptor interaction with radioligand binding experiments [31]. Furthermore, in the same cell line, we found a reciprocal antagonistic A_{2A}-D_2 receptor interaction by which the D_2 receptor, which can couple to $G_{i/o}$ proteins, inhibits the effects of A_{2A} receptor stimulation at the level of adenylyl cyclase [31] (Fig. **2b**). A D_2 receptor agonist did not produce a significant effect on its own, but it completely counteracted the effect induced by A_{2A} receptor stimulation on cAMP accumulation, CREB phosphorylation and *c-fos* expression [31].

The two kind of reciprocal antagonistic A_{2A}-D_2 receptor interactions could also be demonstrated in another cell line, a human SH-SY5Y neuroblastoma cell line that constitutively expresses A_{2A} receptors and with transfected D_2 receptors [32]. In this cell line, D_2 receptor stimulation completely counteracted cAMP accumulation induced by an A_{2A} receptor agonist [8]. But, at the same time, an antagonistic A_{2A}-D_2 intramembrane receptor interaction with functional consequences could be demonstrated with radioligand binding experiments and intracellular Ca^{2+} responses. Thus, D_2 receptor activation inhibited a KCL-induced increase in intracellular concentration of Ca^{2+}, which was counteracted by A_{2A} receptor stimulation [32]. This is most probably the same mechanism by which the antagonistic A_{2A}-D_2 intramembrane receptor interaction controls the excitability of the GABAergic enkephalinergic neurons (see above).

It is intriguing that both types of reciprocal antagonistic A_{2A}-D_2 receptor interactions coexist in the same cells and, in fact, they do coexist in the brain. Under normal conditions, there is a strong tonic activation of D_2 receptors that blocks the ability of A_{2A} receptors to signal through the cAMP-PKA pathway. For instance, in the rodent striatum, the *in vivo* administration of D_2 receptor antagonists produces a significant increase in the PKA-dependent phosphorylation of DARPP-32 or the AMPA receptor and an increase in the expression of *c-fos* and *preproenkephalin* genes, which depends on the ability of D_2 receptor blockade to liberate A_{2A} receptor signaling activated by endogenous adenosine [45,46]. Thus, the effect of the D_2 receptor antagonists was counteracted by the previous administration of an A_{2A} receptor antagonist, which did not have a significant effect on its own [45,46].

COEXISTENCE OF THE RECIPROCAL ANTAGONISTIC A_{2A}-D_2 RECEPTOR INTERACTIONS

There is therefore evidence for the coexistence of two reciprocal antagonistic interactions between A_{2A} and D_2 receptors in the GABAergic enkephalinergic neurons. There is an antagonistic A_{2A}-D_2 intramembrane receptor interaction, which depends on A_{2A}-D_2 receptor heteromerization, which modulates neuronal excitability and neurotransmitter release; and there is an antagonistic A_{2A}-D_2 receptor interaction at the level of adenylyl-cyclase that modulates protein phosphorylation and gene expression. These results provide a clear example of a functional dissociation between neuronal excitability and gene expression. Thus, co-stimulation of A_{2A} and D_2 receptors implies a simultaneous A_{2A} receptor-mediated inhibition of the D_2 receptor-mediated modulation of neuronal excitability and a D_2 receptor-mediated inhibition of the A_{2A} receptor-mediated modulation of gene expression.

There are at least two possible, but not exclusive, mechanisms that could explain this apparently incompatible coexistence of reciprocal antagonistic A_{2A}-D_2 receptor interactions. First, one possibility is a different G-protein coupling between different sets of D_2 receptors. Thus, it has been shown that D_2 receptor couples to $G_{i/o}$, and therefore negatively to adenylyl-cyclase, when not forming heteromers, and that it couples to $G_{q/11}$-PLC signaling when forming heteromers with D_1 receptors [47]. In fact, A_{2A} receptor is morphologically and functionally very similar to the D_1 receptor. They both have a short third intracellular loop and a long acidic C-terminus and they both couple to G_{olf} proteins in the striatum. Also, they most probably use the same epitope (phosphorylated serine in the C-terminus) for their physical interaction with the D_2 receptor [27]. Furthermore, the inhibitory role of D_2 receptors in the excitability of GABAergic enkephalinergic neurons depends mostly on the suppression of Ca^{2+} currents through L-type voltage-dependent calcium channels, which depends on activation of the $G_{q/11}$-PLC signaling pathway [37].

As shown in Fig. (**2**), the most probable scenario is the one that considers that, when not forming heteromers, the most common basic composition of A_{2A} and D_2 receptors and any GPCR is as homodimers [25,48-52], and that only one (heterotrimeric) G protein binds to a receptor dimer [48-50]. The selectivity of G protein recognition is determined by multiple intracellular regions, with the

most critical regions being the second intracellular loop (I2L), the NI3L and the C-terminal segment of the third intracellular loop [53]. The relative contribution of these intracellular receptor domains to the selectivity of G protein recognition varies among different clases of GPCRs [53]. For D_2 receptors, the arginine-rich epitope of the NI3L has been shown to be fundamental for the coupling to $G_{i/o}$ proteins [54]. The same epitope has been demonstrated to bind to calmodulin and, as mentioned before, to the C-terminus of the A_{2A} receptor [10,26,27,55,56]. These findings would agree with the inability of D_2 receptor heteromers to signal through $G_{i/o}$ proteins when bound to A_{2A} receptors (see above) or to calmodulin [55].

Another possibility for the coexistence of reciprocal antagonistic A_{2A}-D_2 receptor interactions is the existence of an additional partner for A_{2A} and D_2 receptors to interact at the adenylyl-cyclase level. A_{2A} receptors have also been found to form receptor heteromers with metabotropic glutamate $mGlu_5$ receptors both in transfected cells and in the striatum [7]. In transfected cells and in the striatum, co-stimulation of A_{2A} and $mGlu_5$ receptors produced a very synergistic effect on *c-fos* expression, which depends on interactions between both receptors at the adenyl-cyclase and at the MAPK levels [7,57]. *In vivo* experiments demonstrated that co-stimulation of A_{2A} and $mGlu_5$ receptors, with the central administration of selective agonists, allows A_{2A} receptor to get rid of the tonic inhibitory effect of D_2 receptor and signal through cAMP-PKA pathway [7]. Since these A_{2A}-$mGlu_5$-D_2 receptor interactions can be demonstrated in animal models of Parkinson's disease [58,59], we postulated that co-administration of A_{2A} and $mGlu_5$ receptor antagonists could be used as a therapeutic strategy in this disease [58]. Similarly, *in vivo* microdialysis experiments have shown that A_{2A}-$mGlu_5$-D_2 receptor interactions modulate the function of the GABAergic enkephalinergic neurons of the nucleus accumbens [60], which can have implications for schizophrenia and drug addiction.

THE SYNERGISTIC A_{2A}-D_2 RECEPTOR INTERACTION AT THE G PROTEIN LEVEL

The antagonistic A_{2A}-D_2 receptor interaction at the adenylyl cyclase level just described depends on the ability of activated D_2 receptors to counteract A_{2A} receptor-mediated type V adenylyl-cyclase (ACV) activation [61]. Under some conditions, a synergistic A_{2A}-D_2 receptor interaction can also be detected [34,62], which seems to depend on the presence of an activator of G protein signaling (AGS3), which facilitates a synergistic interaction between $G_{s/olf}$- and $G_{i/o}$-coupled receptors on the activation of types II/IV adenylyl cyclase (ACII/IV) (reviewed in ref. 63). AGS3 binds preferentially to $G_i\alpha$, and stabilizes the GDP-bound conformation of G_i, thereby dampening the signaling of the receptor through G_i-GTP, while simultaneously increasing the activity of $G\beta\gamma$-regulated effectors [64]. Upon co-activation of the G_i-coupled D_2 receptor, unbound $\beta\gamma$ subunits released in the presence of AGS3 are free to transiently stimulate ACII/IV upon co-activation of the G_{olf}-coupled A_{2A} receptor, leading to a paradoxical increase in cAMP-PKA signaling [63] (Fig. **2c**).

The antagonistic A_{2A}-D_2 receptor interaction at the adenylyl-cyclase is however predominant in most conditions, since ACV is the most expressed type of adenylyl-cyclase in the striatum [65]. However, the synergistic interaction can become particularly important during conditions of upregulation of AGS3, such as during chronic treatment with addictive drugs. It has recently been shown that withdrawal from repeated treatment with cocaine (self- or non-self-administered) up-regulates AGS3 in the prefrontal cortex and in the core region of the nucleus accumbens [66]. In rats, knocking down AGS3 expression in the prefrontal cortex or the nucleus accumbens core (with antisense oligonucleotides) counteracts reinstatement of cocaine- or heroin-seeking behaviour, respectively [66,67]. Therefore, upregulation of AGS3, with the consequent dampening of $G_i\alpha$ signaling while simultaneously promoting $\beta\gamma$-dependent signaling of G_s-coupled receptors, such as D_1 in the prefrontal cortex or in the nucleus accumbens [66], or A_{2A} in the nucleus accumbens [63], can be an important mechanism responsible for the pathophysiologic changes associated with different addictive drugs. Thus, we have postulated that A_{2A} receptor antagonists could be useful in the treatment of drug addiction and relapse during drug withdrawal [63].

PRESYNAPTIC ANTAGONISTIC A_{2A}-D_2 RECEPTOR INTERACTIONS

The GABAergic enkephalinergic neuron does not only express A_{2A} and D_2 receptors in the somatodendritic area, but both receptors are also colocalized in the nerve terminals [16]. Stimulation of A_{2A} receptors in the globus pallidus, localization of the nerve terminals of the GABAergic enkephalinergic neurons, has been shown to stimulate GABA release by using *in vivo* microdialysis and slice preparations [68-70]. This effect of A_{2A} receptor stimulation is dependent on the activation of the cAMP-PKA pathway [70], which depends on the already reported ability of PKA to phosphorylate different elements of the machinery involved in vesicular fusion [71]. In fact, the ability of A_{2A} receptors to stimulate neurotransmitter release through cAMP-PKA signaling has also been demonstrated for acetylcholine in the striatum [72] and serotonin in the hippocampus [73]. In the globus pallidus, stimulation of D_2 receptors produces a strong counteraction of A_{2A} receptor-mediated GABA release [68,70]. Altogether, this has all the characteristics of the antagonistic A_{2A}-D_2 receptor interaction at the second messenger level described above, which does not seem to depend on A_{2A}-D_2 receptor heteromerization. Recent studies in an animal model of Parkinson's disease suggest that this pallidal A_{2A}-D_2 receptor interaction may contribute to the antiparkinsonian effects of the coadministration of A_{2A} antagonists and L-DOPA or D_2 receptor agonists [74].

Finally, there is still another presynaptic localization where A_{2A}-D_2 receptor interactions seem to have an important functional role. We have recently demonstrated that A_{2A} receptors localized in striatal glutamatergic terminals play a very important control of striatal glutamate release [12]. These receptors form heteromers with A_1 receptors, and the A_1-A_{2A} receptor heteromer constitutes a "concentration-dependent switch" that regulates glutamate release depending on the extracellular concentration of adenosine. Thus, low concentrations of adenosine inhibit glutamate release by stimulating the A_1 receptor, while higher concentrations induce glutamate release by also stimulating A_{2A} receptors, which shuts down A_1 receptor signaling by means of an antagonistic A_1-A_{2A} intramembrane receptor interaction [3,12,14,16,17]. However, the A_{2A} receptor-dependent modulation of glutamate release seems to be under an inhibitory control by a co-localized D_2 receptor. Thus, dopamine denervation strongly potentiates A_{2A} receptor agonist-mediated stimulation of striatal glutamate release [71]. Again, this has the biochemical characteristics of an antagonistic A_{2A}-D_2 receptor interaction at the second messenger level, which might not depend on A_{2A}-D_2 receptor heteromerization or which might depend on A_{2A}-$mGlu_5$-D_2 receptor interactions (see above). In fact, A_{2A} and $mGlu_5$ receptors have been found to be co-localized in a large proportion of striatal glutamatergic terminals, where they facilitate glutamate release in a synergistic manner [76]. This presynaptic antagonistic A_{2A}-D_2 receptor interaction can also have implications for the treatment of Parkinson's disease with the co-administration of A_{2A} antagonists and L-DOPA or D_2 receptor agonists. Thus, in the striatum of parkinsonian animals there is overactivity of striatal glutamatergic transmission, which is restored with L-DOPA treatment. In recent electrophysiological experiments in cortico-striatal slices, concomitant activation of D_2 receptors and inactivation of A_{2A} receptors has shown to produce a very significant decrease in striatal glutamate release [77].

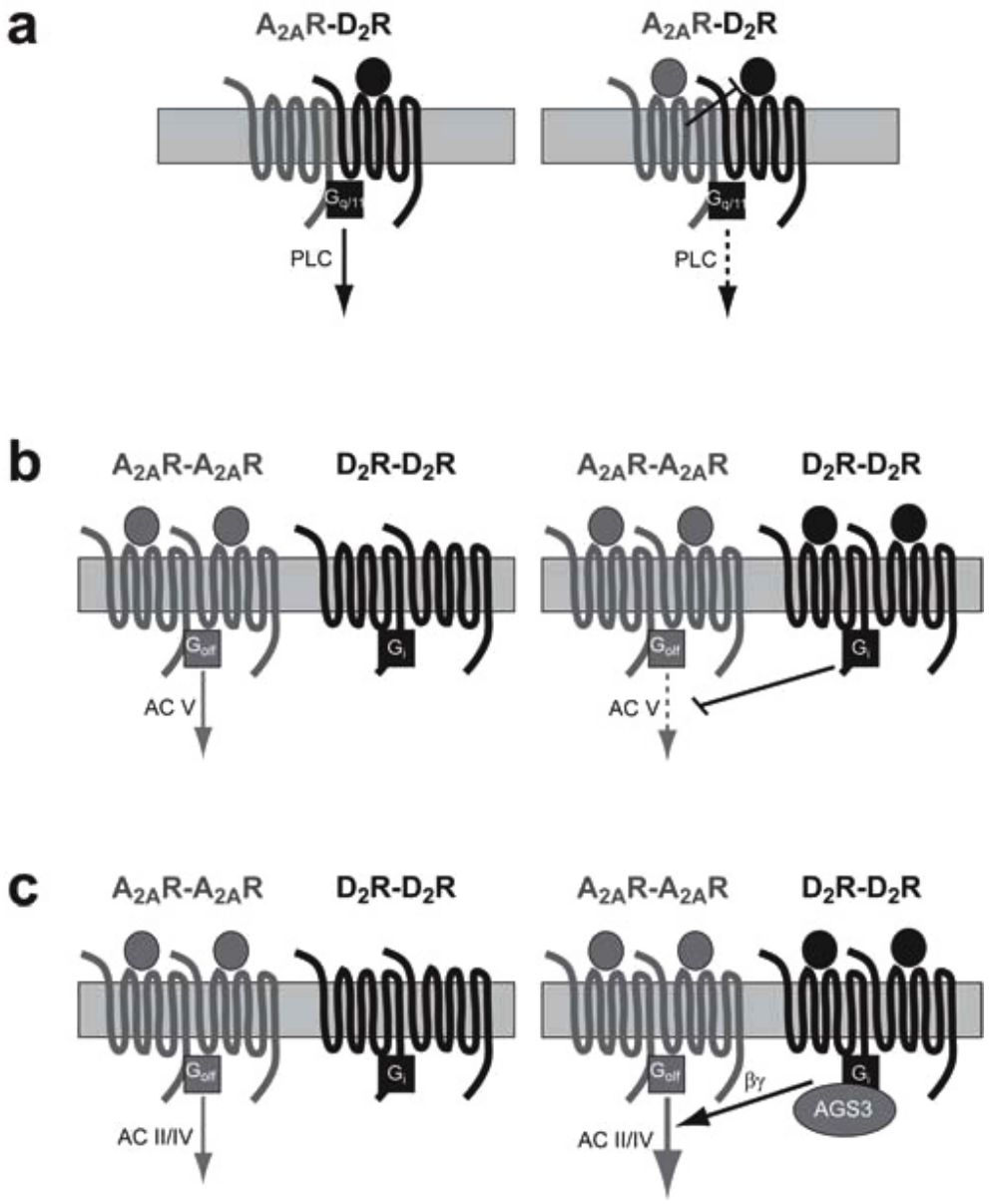

Fig. (2). Different types of A_{2A}-D_2 receptor interactions: **a.** antagonistic A_{2A}-D_2 intramembrane receptor interaction which depends on A_{2A}-D_2 receptor heteromerization and $G_{q/11}$-PLC signaling; **b.** antagonistic A_{2A}-D_2 receptor interaction at the adenylyl-cyclase level, which depends on $G_{s/olf}$- and G_i- type V adenylyl-cyclase signaling; **c.** synergistic A_{2A}-D_2 receptor interaction at the adenylyl-cyclase level, which depends on upregulation of AGS3 and $G_{s/olf}$ - and G_i- type II/IV adenylyl-cyclase signaling.

CONCLUSIONS

In conclusion, we are beginning to understand the complexities of A_2-D_2 receptor interactions, which play a very important role in basal ganglia physiology. Furthermore, it is obvious that the analysis of the different A_{2A}-D_2 receptor interactions will have important general implications about the processing of information of GPCRs. The present review stresses the fact that multiple and functionally different interactions can occur between just two different GPCRs, sometimes involving receptor heteromerization. Particularly important is the example of the coexistence of two apparently incompatible reciprocal antagonistic interactions between A_{2A} and D_2 receptors, which allows a segregated control of neuronal excitability and gene expression in the GABAergic enkephalinergic neurons by the same receptors. The analysis of A_2-D_2 receptor interactions will also have implications for the pathophysiology and treatment of basal ganglia disorders and drug addiction, in view of their key processing role in the computation of information by the striatal spine module [16].

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