Receptor heteromerization in adenosine A_{2A} receptor signaling

Relevance for striatal function and Parkinson's disease

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Abstract—Recently evidence has been presented that adenosine A_{2A} and dopamine D_2 receptors form functional heteromeric receptor complexes as demonstrated in human neuroblastoma cells and mouse fibroblast Ltk^- cells. These A_{2A}/D_2 heteromeric receptor complexes undergo coaggregation, cointernalization, and codesensitization on D_2 or A_{2A} receptor agonist treatments and especially after combined agonist treatment. It is hypothesized that the A_{2A}/D_2 receptor heteromer represents the molecular basis for the antagonistic A_{2A}/D_2 receptor interactions demonstrated at the biochemical and behavioral levels. Functional heteromeric complexes between A_{2A} and metabotropic glutamate 5 receptors (mGluR5) have also recently been demonstrated in HEK-293 cells and rat striatal membrane preparations. The A_{2A}/mGluR5 receptor heteromer may account for the synergism found after combined agonist treatments demonstrated in different in vitro and in vivo models. D_2, A_{2A}, and mGluR5 receptors are found together in the dendritic spines of the striatopallidal GABA neurons. Therefore, possible D_2/A_{2A}/mGluR5 multimeric receptor complexes and the receptor interactions within them may have a major role in controlling the dorsal and ventral striatopallidal GABA neurons involved in Parkinson's disease and in schizophrenia and drug addiction, respectively.

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The A_{2A}/D_2 heteromeric receptor complex. Evidence has accumulated that intramembrane antagonistic receptor–receptor interactions between adenosine A_{2A} and dopamine D_2 receptors exist in dorsal and especially in the ventral striatum as studied in biochemical and receptor autoradiographic experiments.[1-4] Both receptors are located on the dendritic spines of the striatopallidal GABA neurons.[5,6] In 2002, it became possible to demonstrate the existence of A_{2A}/D_2 heteromeric receptor complexes in membrane preparations from human D_2 receptor (long-form) stably transfected SH-SY5Y neuroblastoma cells and from mouse fibroblast Ltk^- cells stably transfected with human D_2 (long-form) receptors and transiently cotransfected with the A_{2A} receptor double tagged with hemagglutinin.[7] It was observed that the A_{2A}/D_2 heteromeric receptor complexes existed in the absence of exogenous A_{2A} and D_2 receptor agonists and therefore represented constitutive heteromers. Experiments using bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) techniques indicate that the A_{2A}/D_2 heteromeric receptor complex represents a heterodimer (Canals et al., unpublished observations).

Based on these observations, it is likely that the A_{2A} receptor agonist-induced reduction of D_2 receptor affinity, mainly involving the high-affinity state, is caused by an activation of the A_{2A} receptor in the heteromeric receptor complex causing a conformational change in the binding pocket of the D_2 receptor.[8] A_{2A} receptor activation also leads to a reduction of the G-protein coupling of the D_2 receptor as seen from an antagonism of the GTP-induced cross-regulation of the D_2 receptor with a disappearance of the high-affinity state.[9] These events result in a reduction of D_2 receptor signaling as inferred from the ability of A_{2A} receptor agonists to counteract the reduction of adenylyl cyclase activity[7] and the changes in intracellular Ca^{2+} levels[8,10] induced by D_2 receptors. Conversely, D_2 receptor activation antagonizes A_{2A} receptor signaling by a G_i-mediated inhibition of the A_{2A}/G_{olf}-activated adenylyl cyclase, which seems to be particularly pronounced in the dorsal striatum.[11] The existence of the A_{2A}/D_2 heteromeric receptor complex is probably also the molecular

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mechanism underlying the demonstrated coaggregation, cointernalization, and codesensitization of A_{2A} and D_2 receptors on A_{2A} or D_2 receptor agonist treatments.[7] It is of substantial interest that combined D_2 and A_{2A} receptor agonist treatment markedly enhances the cointernalization and codesensitization of the A_{2A} and D_2 receptors. Therefore, the A_{2A}/D_2 heteromeric receptor complex may make A_{2A}/D_2 receptor cotrafficking possible.

Relevance of the A_{2A}/D_2 heteromeric receptor complex for PD and its management. Based on the above, it seems likely that the recently demonstrated antiparkinsonian actions of A_{2A} receptor antagonists in humans (see Chase, page S107) are to a substantial degree caused by blocking the action of endogenous adenosine on A_{2A} receptors of the A_{2A}/D_2 receptor heteromer, leading to enhancement of D_2 receptor signaling.[1,3] This may permit the reduction of the L-dopa dose and thus reduces the development of the L-dopa–induced dyskinesias related to a change in the phenotypic character of striatal GABAergic neurons with overexpression of prodynorphin and glutamic acid decarboxylase (GAD) mRNA levels[12] (see Chen et al., page S74).

Carta et al.[13] (see Carta et al., page S39) have shown that combined treatment with an A_{2A} receptor antagonist and a low dose of L-dopa did not produce the possibly deleterious long-term increases in GAD, dynorphin, and enkephalin mRNA levels. By contrast, repeated treatment with a higher dose of L-dopa alone (which produced the same acute motor stimulant effect as did the combination of L-dopa plus A_{2A} antagonist) led to a significant increase in striatal GAD, dynorphin, and enkephalin expression. This absence of striatal gene inductions with repeated L-dopa plus A_{2A} antagonist was correlated with a stable turning response, in contrast to the sensitized turning response that developed after repeated treatment with L-dopa alone in this hemiparkinsonian model in rats.[13] It is of note that A_{2A} receptor antagonists alone produce antiparkinsonian effects without dyskinesias in parkinsonian monkeys.[14-16] It has also been indicated that long-term L-dopa therapy requires A_{2A} receptors for persistent behavioral sensitization as studied in A_{2A} receptor knockout mice.[17]

The loss of inhibition of A_{2A} receptor signaling by the reduced D_2 receptor signaling in patients with Parkinson's disease (PD) adds to the parkinsonian symptoms, and thus antiparkinsonian actions of A_{2A} receptor antagonists can be related not only to an enhancement of D_2 receptor signaling but also to the blockade of increased A_{2A} receptor signaling of the hypodopaminergic state.[11,18] A_{2A} receptor antagonists can counteract motor inhibitory and cataleptic effects after genetic and pharmacologic disruptions of D_2 receptor-mediated transmission.[19,20]

Data on A_{2A}/D_2 receptor cotrafficking[7] suggest that increased A_{2A}/D_2 receptor cointernalization in response to long-term L-dopa therapy, in combination with increased striatal adenosine tone,[7] may contribute to the deterioration of the therapeutic action of L-dopa. Simply stated, the desensitization may result from a decreased membrane presence of the D_2 receptor.

There is also the possibility that A_{2A} receptor antagonists can show neuroprotective activity because a higher coffee and caffeine intake was associated with reduced risk for PD.[21,22] A_{2A} receptor antagonists may reduce excitotoxicity[23] because, for example, the stimulation of striatal glutamate release by metabotropic glutamate 5 receptor (mGluR5) agonists involves A_{2A} receptors.[24]

In our opinion, an important aspect to be further investigated to reach a better understanding of the sensitization/desensitization process of the D_2 receptors may reside in the stoichiometry of the A_{2A}/D_2 receptor heterodimers vs the A_{2A} and D_2 receptor monomers and homodimers present at the plasma membrane level. In particular, long-term L-dopa therapy may induce internalization of A_{2A} and D_2 receptors when associated as heterodimers, whereas it may not affect A_{2A} receptors existing as monomers or homodimers.[7] Therefore, the relative amount of D_2 receptors in the two forms may be of importance in controlling the manifold aspects (efficacy and potency of D_2 signaling trafficking, sensitization, and desensitization) of the neuronal response to long-term L-dopa therapy.

The A_{2A}/mGluR5 heteromeric receptor complex. Our interest in A_{2A}/mGluR5 receptor interactions started with the demonstration that A_{2A} and group I mGluR receptor agonists could synergistically reduce affinity of D_2 receptor in striatal membranes.[25] Recently it has been possible to show the existence of heteromeric receptor complexes between A_{2A} receptors and the group I mGluR receptor subtype mGluR5 in coimmunoprecipitation experiments in HEK-293 cells cotransfected with Flag-A_{2A} and hemagglutinin mGluR5 receptors and in rat striatal membrane preparations involving native A_{2A} and mGluR5 receptors.[26] In contrast, there was a lack of coimmunoprecipitation between mGluR5 and mGluR1β (an isoform of another group I mGluR receptor) receptors. In agreement, it was found that the A_{2A} and mGluR5 receptors were colocalized on the membrane surface of cotransfected HEK-293 cells as shown with confocal laser microscopy after transient cotransfections. Furthermore, in preliminary fluorescence microscopy experiments with optical sectioning techniques (using the exhaustive photon reassignment process) we have found evidence for a strong A_{2A}/mGluR5 receptor colocalization in rat striatal primary cultures (figure). The detailed quantitative analysis of these results is in preparation (only the images corresponding to the pictures in the figure have been analyzed). It is presently unknown if adapter proteins, such as Homer proteins or the Shank family of scaffold proteins, link mGluR5 and A_{2A} receptors together in the het-

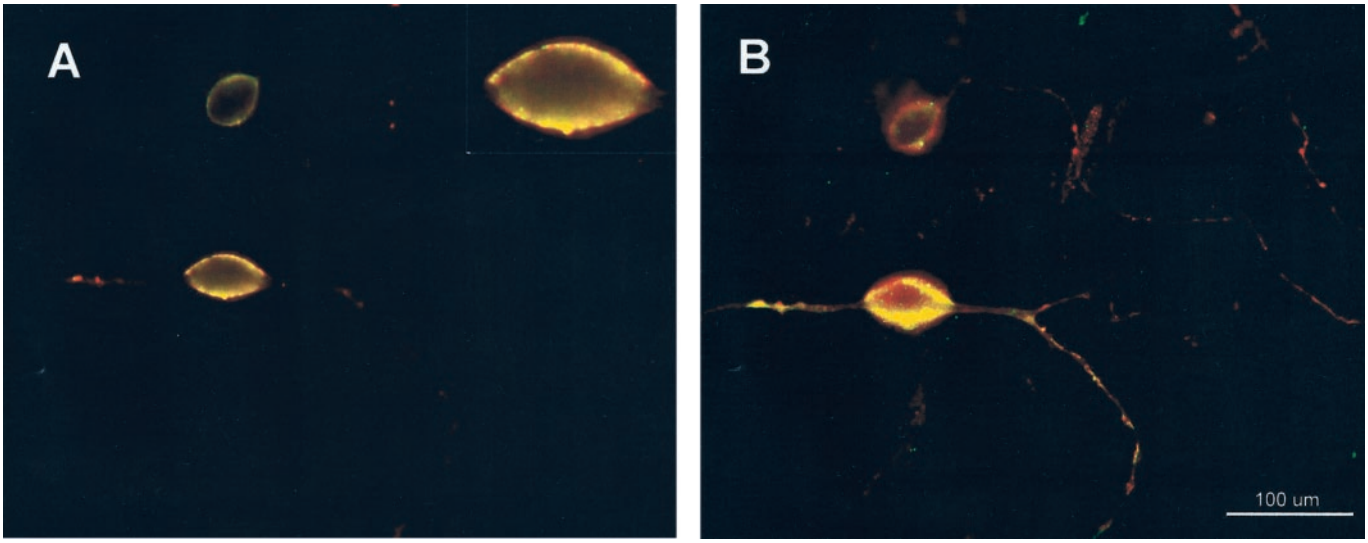

Figure. Colocalization of A_{2A} and metabotropic glutamate 5 (mGluR5) receptor immunoreactivities in the soma (A) and soma and dendrites (B) of striatal neurons in primary cultures (day 15 in vitro). Cryopreserved primary striatal neurons were obtained from QBM Cell Science (R-Cp-502; Ottawa, Canada). Cells were stored in liquid nitrogen until use. Cells were immunostained with rabbit anti-mGluR5 receptor (1:100; Upstate Biotechnology, Lake Placid, NY) and mouse anti-A_{2A} receptor (1:1000) antibodies.[40] Goat antirabbit Alexa-Fluor 488 (1:400; Molecular Probes, Eugene, OR) and goat anti-mouse CY3 (1:400; Sigma Chemical Co., St. Louis, MO) conjugated antibodies were used as secondary antibodies. The immunostaining procedure is the same as described in detail elsewhere for permeabilized cells[41] (the A_{2A} receptor antibody is directed against an intracellular epitope[40]) with some modifications. Cells were analyzed by double immunofluorescence with confocal-like microscopy. Superimposition of the red (CY3) and green (Alexa-Fluor 488) images reveals the A_{2A}/mGluR5 receptor colocalization in yellow (magnification bar, 100 μm). The "Boolean colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors) at soma level was equal to approximately 33% of the entire soma field area, whereas the "yellow colocalization" (overlap of the field areas between A_{2A} and mGluR5 receptors where red and green emission showed similar intensity of emission) at soma level was equal to approximately 4.5% of the Boolean colocalization. This yellow colocalization most probably represents the area with the highest ratio of A_{2A}/mGluR5 receptor heteromeric complexes vs A_{2A} and mGluR5 homomeric (or monomeric) receptor complexes (for detailed description of the method, see elsewhere[42]).

eromeric receptor complex. The Homer proteins can bind to the C-terminal part of mGluR5 receptor and produce their clustering, and the Shank proteins link together the mGluR5 with the NMDA receptors. The A_{2A}/mGluR5 receptor heteromer was also present in the absence of exogenous agonists and appeared to be preformed, like the A_{2A}/D_2 receptor heteromer.

In the HEK-293 cells, it was possible to give a functional correlate to the A_{2A}/mGluR5 heteromeric receptor complex.[26] Therefore, A_{2A}/mGluR5 receptor coactivation produced a synergistic interaction at the level of extracellular signal-regulated kinase 1/2 (ERK) phosphorylation and c-fos expression. These and other results suggested that the A_{2A}/mGluR5 heteromeric receptor complex is involved in striatal neuron plasticity, including long-term potentiation and depression.[26] This synergism may be brought about by independent signals interacting at the level of the mitogen-activated protein kinase (MAPK) cascade.[27] There is also the possibility that on coactivation of the heteromeric receptor complex, a multireceptor complex may be assembled with receptor tyrosine kinases or nonreceptor tyrosine kinase Src, leading to ERK activation.[28-32] Another possible mechanism involved in the synergism between A_{2A} and mGluR5 receptors could be the modulation of mGluR5 receptor desensitization, which has been demonstrated for NMDA/mGluR5 and group II mGluR/mGluR5 receptor interactions.[33,34] In these cases, the modulation seems to depend on the activation of phosphatase 2B, which reverses the agonist-induced protein kinase C-mediated desensitization of mGluR5 receptors.[33,34] However, this mechanism would imply synergistic interactions at the second-messenger level (Ca^{2+} mobilization),[33,34] which could not be demonstrated in A_{2A}/mGluR5 receptor-cotransfected HEK-293 cells.[26]

A similar A_{2A}/mGluR5 receptor synergism could be demonstrated in the rat striatum for c-fos expression correlated with a synergistic A_{2A}/mGluR5-mediated counteraction of phencyclidine-induced motor activity.[26] It is well known that this motor activity depends on D_2 receptor activity and has so far been blocked only by a high degree of D_2 receptor blockade. Therefore, the results suggest that A_{2A}/mGluR5 receptor costimulation can override a strong D_2 receptor-mediated transmission at the behavioral level. Long-term, but not short-term, treatment with an mGluR5 receptor antagonist can reverse the akinetic deficit in a model of PD.[35] It seems possible that this can in part be caused by an internalization and downregulation of the A_{2A}/mGluR5 heteromeric re-

ceptor complex removing the D_2 receptor from inhibition of its signaling. In addition, there may also exist an acute mGluR5 receptor antagonist-induced counteraction of D_2 receptor blockade with haloperidol as seen from reduced rigidity and catalepsy[36] that may in part be exerted at the network level (e.g., at the level of the subthalamic nigral glutamate system).

The possible existence of A_{2A}/D_2/mGluR5 multimeric receptor complexes. The A_{2A}/mGluR5 heteromeric receptor complexes appear to be preferentially located at dendritic spines of striatopallidal GABAergic neurons[20,37] like the A_{2A}/D_2 heteromeric receptor complexes. Based on the aforementioned observations, it seems a reasonable hypothesis that there exist A_{2A}/D_2/mGluR5 multimeric receptor complexes in the dendritic spines of the striatopallidal GABA neurons.

Observations supporting this hypothesis:

1. A_{2A} and group I mGluR synergistically reduced the affinity of the high-affinity state of the striatal D_2 receptors in membrane preparations.[25]
2. Group I mGluR activation synergistically potentiated the ability of the A_{2A} receptor agonist CGS21680 to counteract D_2 receptor agonist (quinpirole)-induced contralateral rotational behavior.[25]
3. The mGluR5 receptor agonist CHPG reduced the affinity of the high-affinity state of the D_2 receptor, an action potentiated by CGS21680.[38]
4. CHPG inhibited contralateral rotational behaviors induced by quinpirole, an effect potentiated by CGS21680.[38]
5. The mGluR5 receptor agonist CHPG in the nucleus accumbens increased GABA release in the ventral pallidum, an action strongly potentiated by coperfusion with CGS21680.[39]
6. Coperfusion with quinpirole counteracted the increases in ventral pallidal GABA levels by CGS21680 and CHPG.[39]

These results are compatible with the existence of A_{2A}/D_2/mGluR5 multimeric complexes that are important in regulation of the dorsal and ventral striatopallidal GABA neurons and are of high relevance for management of PD (dorsal system) and schizophrenia and drug dependence (ventral system).

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