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A Two-state Model for the Diffusion of the A_{2A} Adenosine Receptor in Hippocampal Neurons

*AGONIST-INDUCED SWITCH TO SLOW MOBILITY IS MODIFIED BY SYNAPSE-ASSOCIATED PROTEIN 102 (SAP102)**

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Background: Agonist activation slows diffusion of the A_{2A} receptor in the lipid bilayer.
Results: In hippocampal neurons, the agonist-induced decrease in mobility was accounted for by both the hydrophobic receptor core and its extended C terminus, which recruited SAP102.
Conclusion: The observations are consistent with two diffusion states of the A_{2A} receptor in neurons.
Significance: SAP102 regulates access of the A_{2A} receptor to a compartment with restricted mobility.

The A_{2A} receptor is a class A/rhodopsin-like G protein-coupled receptor. Coupling to its cognate protein, G_s, occurs via restricted collision coupling and is contingent on the presence of cholesterol. Agonist activation slows diffusion of the A_{2A} adenosine receptor in the lipid bilayer. We explored the contribution of the hydrophobic core and of the extended C terminus by examining diffusion of quantum dot-labeled receptor variants in dissociated hippocampal neurons. Single particle tracking of the A_{2A} receptor(1–311), which lacks the last 101 residues, revealed that agonist-induced confinement was abolished and that the agonist-induced decrease in diffusivity was reduced substantially. A fragment comprising the SH3 domain and the guanylate kinase domain of synapse-associated protein 102 (SAP102) was identified as a candidate interactor that bound to the A_{2A} receptor C terminus. Complex formation between the A_{2A} receptor and SAP102 was verified by coimmunoprecipitation and by tracking its impact on receptor diffusion. An analysis of all trajectories by a hidden Markov model was consistent with two diffusion states where agonist activation reduced the transition between the two states and, thus, promoted the accumulation of the A_{2A} receptor in the compartment with slow mobility. Overexpression of SAP102 precluded the access of the A_{2A} receptor to a compartment with restricted mobility. In contrast, a mutated A_{2A} receptor (with ^{383}DVELL^{387} replaced by RVRAA) was insensitive to the action of SAP102. These observations show that the hydrophobic core *per se* does not fully account for the agonist-promoted change in mobility of the A_{2A} receptor. The extended carboxyl terminus allows for regulatory input by

Adenosine is the metabolite of last resort. Cell damage an hypoxia lead to its extracellular accumulation. As a conse quence, four G protein-coupled receptors (termed A_1, A_{2A} A_{2B}, and A_3) are activated. The combined action of these fou receptors is to orchestrate short- and long-term responses tha further mitigate cell damage and activate tissue repair (1). Thi retaliatory metabolite concept can be exemplified by consider ing the role of the G_s-coupled A_{2A} receptor in the vasculature where short-term activation leads to vasodilation and increase blood supply, and long-term activation causes endothelial ce proliferation (2). In addition, adenosine is formed by th sequential action of ectonucleotidases from ATP that is store in and released from synaptic vesicles (3). Thus, in the nervou system, adenosine acts as a neuromodulator. In the indirec pathway of the basal ganglia, for instance, volume transmissio by adenosine engages striatal A_{2A} receptors and determines th set point for wired transmission (4). Accordingly, genetic dele tion of A_{2A} receptors in the brain has complex phenotypic cor sequences, depending on whether the receptor is deleted glob ally or eliminated from specific regions (5). The psychomoto stimulant action of cocaine, for instance, is enhanced by abla tion of the A_{2A} receptor in the striatopallidal projection neu rons but blunted by elimination in the forebrain (6).

The A_{2A} adenosine receptor has several unique propertie when compared with its closest relatives (*i.e.* the other adeno sine receptors) and to the entire class of class A/rhodopsin-lik GPCRs[3] (7). Its C terminus is very long (122 amino acids) and thus, provides docking sites for interacting proteins (8). It lack