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MEETING ABSTRACT

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Analyzing the role of diffusion for A_{2A} receptor function in hippocampal neurons

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Background: The adenosine A_{2A} receptor is a classical G protein-coupled receptor and has a very long carboxyl terminus that lacks the canonical palmitoylation site. In addition, the A_{2A} receptor differs in the mode of G protein activation. Coupling to its cognate protein G_s occurs via restricted collision coupling and is contingent on the presence of cholesterol. 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.

Methods: We used the technique of "single particle tracking", which has a high spatial and temporal resolution to study receptor diffusion on the cell membrane. We labeled A_{2A} receptors expressed on hippocampal neurons with quantum dots, video-recorded the movement of the fluorescent dots and used computer-assisted detection and diffusional analysis of the obtained trajectories. Finally, we utilized hidden Markov models to further analyze the data.

Results: The analysis of all trajectories revealed two diffusion states of the A_{2A} receptor. Agonist activation reduced the transition between the two states and promoted the accumulation in the state of slow mobility. However, this agonist-induced redistribution was abolished with a truncated A_{2A} receptor, which lacks the last 101 residues, and agonist-induced decrease in diffusivity was substantially reduced. We identified a fragment comprising the SH3 and the guanylate kinase (GUK) domain of synapse-associated protein 102 (SAP102) as a candidate that bound to the A_{2A} receptor C-terminus. The over-expression of SAP102 precluded the access of the A_{2A} receptor to the compartment with reduced mobility. In contrast, an A_{2A} receptor with mutated C-terminus (with ^{383}DVELL^{387} replaced by ^{383}RVRAA^{387}) was insensitive to the action of SAP102.

Discussion: Our observations demonstrate that agonist-induced changes in the hydrophobic core cannot fully explain the change in mobility of the A_{2A} receptor. The extended carboxyl terminus interacts with scaffolding molecules such as SAP102, which has an additional regulatory influence on A_{2A} receptor confinement in neurons.

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