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Meeting abstract

A_{2A} adenosine receptor interacting partners (interactome)

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Background

The A_{2A} adenosine receptor is a member of the G protein-coupled receptor family. It is known to posses several unique structural and functional features that differ from established signaling paradigms. Recently, the exceptionally long (>120 amino acids) C-terminus of the receptor has raised interest for its capacity to act as a binding site for several "accessory" proteins. In search for interaction partners we identified a list of possible candidates via a yeast two-hybrid system. From the identified potential interaction partners we further investigated (i) ARNO/cytohesin-2, a guanine nucleotide exchange factor for the small G protein ARF6, and (ii) SAP102, ''synapse-associated protein of 102 kDa'', and their effects on the signaling properties of the A_{2A} receptor.

Methods and results

Cell-lines (HEK-293 and PC12) with inducible expression of ARNO or its catalytic inactive mutant E156K, as well as cell-lines expressing ARF6 or the dominant negative mutant T27N were created. None of these proteins altered the receptor expression, signaling via adenylyl cyclase after activation or long-term de- and resensitization kinetics in PC12 cells. On the other hand, ARF6 and its guanine nucleotide exchange factor ARNO are recruited to the cell membrane after agonist stimulation of the receptor, where they seem to stabilize the receptor/G protein complex. The mutant proteins are recruited to the membrane in a similar way but do not stabilize the receptor/ G protein complex. SAP102, as its eponymous name suggests, is located at synaptic sites and is known to recruit glutamate receptors during different stages of the brain development. The overexpression of SAP102 in mouse hippocampal neurons showed no apparent effects on the receptor signaling properties. However, the role of SAP102 as an interaction partner may lie in the alteration of receptor mobility. To visualize possible differences in A_{2A} receptor membrane diffusion we utilize a single-particle tracking approach. Epitope-tagged receptors are labeled with quantum dots and their trajectories are recorded under various conditions. To further characterize the A_{2A} receptor interactome under more physiological conditions we use a two-step proteomics approach combining the tandem affinity purification method and 2D-nano-LC-MS/MS. This requires the introduction of an epitope-tagged version of the A_{2A} receptor. After establishing the method in heterologous expression systems we want to continue with this approach in native mouse tissues. Therefore we will generate transgenic mice expressing a tagged A_{2A} receptor.