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Meeting abstract

ARNO/cytohesin-2 regulates desensitization of the A_{2A} adenosine receptor in rat pheochromocytoma cells

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The A_{2A} adenosine receptor is a G protein-coupled receptor which desensitizes upon prolonged agonist stimulation. In order to understand the biological function of its unusually long C-terminus, we screened a human brain library for proteins capable of binding to the last 120 amino acids of the A_{2A} receptor. We identified a guanine nucleotide exchange factor for the small G protein ARF6 (ARNO/cytohesin-2) as a binding partner. In this study, we investigated the impact of ARNO on A_{2A} receptor signaling in rat pheochromocytoma (PC12) cells. These cells express the A_{2A} receptors endogenously. We created cell lines with inducible expression of ARNO or its catalytic inactive mutant E156K. Neither wild type ARNO nor the mutant had an effect on receptor expression, signaling via adenylyl cyclase after activation or long-term de- and resensitization kinetics. In order to investigate effects of ARNO on A_{2A} receptor short-term de- and resensitization we employed a FRET-based sensor to measure changes in cAMP in real time. Cells were transfected with plasmids encoding the regulatory and catalytic subunit of protein kinase A (PKA) fused to CFP and YFP, respectively. Accumulation of cAMP results in the dissociation of the PKA subunits, which can be measured in single cells as a loss of FRET. The presence of dominant negative ARNO accelerated the recovery of A_{2A} receptor after stimulation and led to a pronounced signaling response when cells were re-challenged with agonist. While membrane recruitment of ARNO was not affected by the mutation, we observed a difference in the recovery of the A_{2A} receptor after agonist treatment. Our results indicate that the interaction with ARNO/cytohesin-2 stabilizes short-term desensitization of the A_{2A} receptor to prevent excessive stimulation.

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