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MEETING ABSTRACT

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Functional and physical interactions between P2Y receptors and ion channels

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Background: Neuronal P2Y receptors, *i.e.* nucleotide-sensitive G protein-coupled receptors (GPCRs), are known to control various voltage-gated ion channels, in particular K_V7 potassium and $Ca_V2.2$ calcium channels. The differential modulation of these ion channels via GPCRs was shown to rely on the presence or absence of scaffolding proteins. Since scaffold proteins are believed to bring GPCRs and ion channels in close proximity to guarantee efficient G protein-mediated modulation, this project evaluated whether a tight contact between P2Y receptors and ion channels is a prerequisite for their functional interaction.

Methods: P2Y receptors and ion channels were labeled either with CFP or YFP. For all experiments, a CFP/YFP pair of receptor and channel was transfected transiently into tsA201 cells. Channel modulation by nucleotides was determined by patch-clamp recordings. The fluorescence microscopy techniques FRET (Förster resonance energy transfer) and DRAP (donor recovery after acceptor photobleaching) were used to determine the protein–protein interaction between receptors and channels. Furthermore, FRAP (fluorescence recovery after photobleaching) was performed to elucidate the mobility of the receptors and channels in the membrane.

Results: Activation of $P2Y_1$, but not of $P2Y_{12}$, receptors by ADP inhibited the K^+ currents in a concentration-dependent manner by up to 20.5 ± 1.9%. Conversely, activation of both, $P2Y_1$ and $P2Y_{12}$, receptors reduced the Ca^{2+} currents by up to 60.1 ± 7.4% and 76.3 ± 4.2%, respectively. FRET and DRAP experiments showed that $P2Y_1$ has a protein–protein interaction with both, $K_V7.2/7.3$ (NFRET 0.32 ± 0.02, DRAP recovery 10.3 ± 3.0%) and $Ca_V2.2$ (NFRET 0.37 ± 0.02, DRAP recovery 10.1 ± 1.8%). On the other hand, $P2Y_{12}$ has an interaction only with $Ca_V2.2$ (NFRET 0.39 ± 0.03, DRAP recovery 12.7 ± 1.0%) but not with the $K_V7.2/7.3$ channels. FRAP experiments revealed that the mobility of the ion channels alone is higher than that of the receptors. The coexpression of the P2Y receptors significantly reduced the mobility of the $Ca_V2.2$ channel by 50% (from 3.3 sec to 6.2 sec). In the case of $K_V7.2/7.3$ channels, the τ values were not significantly changed by the presence of P2Y.

Discussion: The functional control of K_V7 by $P2Y_1$ and $Ca_V2.2$ by $P2Y_1$ and $P2Y_{12}$ receptors relies on a close apposition of receptors and channels. In the case of $Ca_V2.2$ and P2Y12 this is even accompanied by a physical interaction.

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