

Meeting abstract

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RIM modulates $\text{Ca}_v1.3$ Ca^{2+} channels

Mathias Gebhart¹, Gabriella Juhasz-Vedres¹, Alexander Trockenbacher¹,
Gerald J Obermair², Jutta Engel³, Alexandra Koschak¹ and Jörg Striessnig^{*1}

Address: ¹Institute of Pharmacy, Pharmacology and Toxicology, Center for Molecular Biosciences, University of Innsbruck, 6020 Innsbruck, Austria, ²Division of Physiology, Innsbruck Medical University, 6020 Innsbruck, Austria and ³Institute of Physiology II and Tübingen Hearing Research Centre, University of Tübingen, 72076 Tübingen, Germany

Email: Jörg Striessnig* - joerg.striessnig@uibk.ac.at

* Corresponding author

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Calcium channel β subunits ($\text{Ca}_v\beta$ s) are essential cytoplasmic components of voltage-gated calcium channels (VGCCs) affecting their gating and targeting. $\text{Ca}_v\beta$ s bind with high affinity to the cytoplasmic loop between transmembrane segments I and II of the $\alpha 1$ subunit (loop-I-II). To identify new proteins that modulate VGCCs by interaction with $\text{Ca}_v\beta$ s we performed a yeast two-hybrid screen using $\text{Ca}_v\beta 2a$ as bait. Screening of a human fetal brain cDNA library identified a C-terminal fragment of RIM1 α (Rab3-interacting molecule) which contains a highly conserved C2B domain as potential interaction partner. To proof the interaction between RIM and $\text{Ca}_v\beta$ s we developed a protein targeting assay in tsA-201 cells heterologously expressing the loop-I-II of $\text{Ca}_v1.3$ channels with diverse $\text{Ca}_v\beta$ subunits. The $\text{Ca}_v1.3$ -loop-I-II was transported to the plasma membrane and co-targeted all $\text{Ca}_v\beta$ subunits indicating that the $\text{Ca}_v1.3$ -loop-I-II and the $\text{Ca}_v\beta$ subunits formed a functional complex. The C-terminal fragment of RIM1 α or the full-length form of RIM2 β exhibited a cytoplasmic distribution but when co-expressed with $\text{Ca}_v\beta$ s in presence of the $\text{Ca}_v1.3$ -loop-I-II both were co-localized at the plasma membrane. Using qualitative RT-PCR analysis we detected various RIM isoforms in the total organ of Corti and RIM2 α in cochlea inner hair cells (IHCs) at an early developmental stage, before the onset of hearing. As RIM is a presynaptic active zone protein involved in Ca^{2+} -induced neurotransmitter release, we asked the question whether the association of RIM with $\text{Ca}_v1.3$ could account for the slow $\text{Ca}_v1.3$ cur-

rent inactivation seen in IHCs. In whole-cell patch-clamp analysis of tsA-201 cells using 15 mM Ca^{2+} as charge carrier the C2B domain containing fragments of RIM1 α and RIM2 α caused a significant depolarizing shift of the activation-curve of $\text{Ca}_v1.3$ (7–12 mV) and slowed the inactivation of both Ca^{2+} and Ba^{2+} currents ($p < 0.05$) albeit to a lesser extent as found in native IHCs. To investigate if a slowly inactivating $\text{Ca}_v1.3$ spliceform (1b) could contribute to this effect we examined its expression with RT-PCR analysis. However, we did not detect $\text{Ca}_v1.3(1b)$ transcripts in the total organ of Corti at the same developmental stage as we found RIM. Taken together these data showed that indeed RIM modulated $\text{Ca}_v1.3$ Ca^{2+} channels. However, we assume that a mixture of diverse proteins and/or $\text{Ca}_v1.3$ splice variants probably accounts for the slow current inactivation of these channels in native IHCs.

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