Ion Channels, other

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The Possible Role of PSD-95 in the Rearrangement of Kv1.3 Channels to the Immunological Synapse

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T lymphocytes play a crucial role in the adaptive immune response. They are activated through an interaction with antigen presenting cells during the establishment of the immunological synapse (IS). It is well documented that several proteins accumulate in the IS. We have previously shown that one of these molecules is the voltage-gated potassium channel, Kv1.3. However, the mechanism of this translocation and its consequences still remain unknown. It has been anticipated that Kv1.3 channels interact with proteins from the MAGUK family: PSD-95 and SAP97. These two proteins could regulate the cell surface expression and phosphorylation of several Kv1 channels. Consequently, we investigated if these proteins were expressed by human T cells and affect the redistribution of Kv1.3 to the IS.

We could detect both PSD-95 and SAP97 in Jurkat cells (mRNA and protein). Furthermore, both proteins co-immunoprecipitated with the Kv1.3 channel, which implies a direct interaction between these proteins. We established Jurkat cell lines that over-express mGFP tagged wild-type or a C-terminal truncated Kv1.3 channel, which lack the canonical binding domain of PSD-95. Also, we knocked down the PSD-95 gene in the mGFP-tagged WT Kv1.3 expressing cells.

We found that the redistribution of Kv1.3 channels exhibits different kinetics in the wild-type and in the truncated ion channel expressing or PSD-95 knockdown cells: wild-type channels accumulated in the contact region between T and B cells even at the first minute, while the C-truncated channels diffused slower to the IS, just like the wild-type channels of the PSD-95 knockdown cells. Moreover, the accumulation of truncated Kv1.3 channels and WT channels expressed by PSD-95 knockdown cells was less pronounced as compared to the wild-type. These results demonstrate that PSD-95 could control the redistribution of Kv1.3 into the IS.

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Microglial Kv1.3 Channels as a Potential Target for Alzheimer's Disease

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Microglia, the major inflammatory cells of the brain, play a pivotal role in the initiation and progression of Alzheimer's Disease (AD) by either clearing amyloid-β deposits through phagocytic activity or by releasing cytotoxic substances and pro-inflammatory cytokines in response to activation by amyloid-β aggregates, including amyloid-β oligomers (AβO). We here propose microglial Kv1.3 channels as a novel pharmacological target for curbing the harmful effects of Aβ-induced microglia activation. Kv1.3 expression increased in cultured microglia in response to stimulation with AβO as determined by electrophysiology, RT-PCR, Western Blotting and immunohistochemistry. Similarly, microglia isolated from the brains of adult 5xFAD mice, a mouse model expressing high levels of Aβ due to trangenes with AD familial mutations, expressed higher levels of Kv1.3 than microglia isolated from the brains of age-matched control mice. We further observed strong Kv1.3 immunoreactivities in microglia associated with amyloid plaques in brains of 5xFAD mice. Proof for the functional importance of Kv1.3 in microglia comes from our observations that the Kv1.3 blocker PAP-1 inhibits AβO-stimulated nitric oxide production as well as microglia-mediated neurotoxicity in dissociated cultures and organotypic brain slices. A 6-week course of daily PAP-1 injections also reduced the degree of microglia activation in 5xFAD mice. In contrast, Kv1.3 blockade with PAP-1 does not affect phagocytosis of Aβ aggregates by microglia. These observations raise the exciting possibility that Kv1.3 blockers might preferentially inhibit microglia mediated neuronal killing without affecting beneficial functions such as scavenging of debris.

Supported by NIH (RO1GM076063, RO1AG010129 and R21AG038910) and Alzheimer's Association (NIRG-10-174150).

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Modeling Block of the Kv1.5 Channel by Cationophilic and Cationic Ligands: Implications for General Mechanisms of the Inner Pore Block in P-Loop Channels

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Many small molecules of intriguingly different chemical structures bind in the inner pore of potassium channels. Previous mutational, electrophysiological, and ligand-binding experiments revealed common and diverse characteristics of action of different ligands including ligand-channel stoichiometries, voltage- and use-dependencies, and patterns of ligand-sensing residues. However, generally accepted structural interpretations for most of the available data are lacking. Here we used energy calculations with experimentally based constraints to dock flecainide, ICAGEN-4, benzocaine, vernakalant, and AVE0118 into the inner pore of the Kv1.5 channel. We arrived at ligand-binding models that for the first time explain: (i) the different Hill coefficients, (ii) opposite voltage dependencies of cationic and electroneutral cationophilic ligands, and (iii) effects of mutations of residues, which do not face the pore, on the ligand action. Two concepts were crucial for the modeling. First, the inner-pore block of a potassium channel requires a cationic ''blocking particle''. A ligand that lacks a positively charged group, blocks the channels not per se, but in a complex with a permeant cation. Second, flexible hydrophobic moieties of ligands have a tendency to escape from the aqueous pore environment into subunit interfaces. Previously these concepts have allowed to explain action of so different ligands of potassium channels as correolide, propafenone, phenylalkylamines, and PAP-1-like immunosuppressants. The same concepts have been successfully used to analyze structure-function relationships of phenylalkylamines, benzothiazepines and dihydropyridines in calcium channels and local anesthetics in sodium channels. Supported by RFBR to DBT and NSERC to BSZ.

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Development of Atomistic Models for Closed, Open and Open-Inactivated States of hERG1 Channel using Rosetta Protein Modeling Suite and Molecular Dynamics Simulations

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The human ether-a-go-go related gene 1 (hERG1) K ion channel is a key element for the rapid component of the delayed rectified potassium current in cardiac myocytes. It is essential for the normal repolarization phase of the cardiac action potential. Loss of function mutations in hERG1 cause increased duration of ventricular repolarization which leads to prolongation of the time interval between Q and T waves of the body surface electrocardiogram (long QT syndrome-LQTS). hERG1 K+ channel is a homotetramer composed of four identical homologous subunits each containing six transmembrane (TM) helices S1-S6. Single hERG channels are either closed, open or inactivated conformations depending on TM voltage. Although creation and validation of reliable 3D atomistic models of the hERG channel has been a key target in molecular cardiology for last decade, there are no reported S1-S6 TM homology models of the channel in different states yet in literature. In this study, we derived open, closed and open-inactivated states of hERG1 using ROSETTA protein modeling suite and side-chain placements are optimized by molecular dynamics simulations. Although backbone templates are modeled on available crystal structures of Kv1.2 and KcsA channels, the missing parts are modeled de novo. Final models are evaluated for consistency to the reported structural elements discovered mainly on the basis of mutagenesis and electrophysiology. Closed state models are further validated by protein-protein docking using selective peptide toxin and available experimental data on toxin foot-printing. Derived models of hERG1 in different states offer an indispensable template for rational drug design as well as better understanding of molecular mechanisms of hERG channel upon binding of openers or blockers.

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Molecular Determinants of Pentamidine-Induced hERG Trafficking Inhibition

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Pentamidine is an anti-protozoical compound that clinically causes acquired long QT syndrome which is associated with prolonged QT intervals, tachycardias and sudden cardiac arrest. Pentamidine delays terminal repolarization in human heart by blocking cardiac inward rectifier currents acutely. At the same time, pentamidine reduces surface expression of the cardiac potassium channel I_{Kr}/hERG. This is unusual in that acLQTS is caused most often by direct block of the cardiac potassium current I_{Kr}/hERG. The present study was designed to provide a more complete picture of how hERG surface expression is disrupted by pentamidine at the cellular and molecular level.