pathological B-cells cultured in the presence of cells mimicking bone marrow environment are in progress.

3740-Pos Board B468

The Sh3-Binding Domain of Kv1.3 Channels is Required for their Cortactin-Conveyed Coupling to Actin

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Kv1.3 channels of T lymphocytes play an important role in several physiological functions like activation or migration, which are accompanied by the redistribution of channels in the cell membrane. Both processes are characterized by F-actin polymerization indicating the involvement of the actin cytoskeleton in the associated channel's redistribution. Several studies reported that Kv1.x channels are linked to or co-localize with various adapter proteins which also possess actin-binding domains: hDlg1 via the PDZ-binding domain and cortactin through either the cortactin- or the SH3-binding site. In this study we investigated the molecular requirements for Kv1.3 channel lateral membrane diffusion.

FLAG/EGFP-tagged Kv1.3 wild-type or mutant constructs were expressed in HEK-293 cells. We designed C-terminal truncated (without (delta1) or with (delta2) cortactin binding sequence, and both without SH3; with cortactin and SH3 binding motif (delta3)) and few-amino-acid Kv1.3 mutants (deltaSH3/deltaPDZ: SH3/PDZ domain ruptured). Biophysical properties of each phenotype were evaluated with patch-clamping. Interaction/co-localization between channel and adaptor proteins was studied with co-immunoprecipitation/confocal microscopy. Lateral mobility of Kv1.3 was assessed with FRAP and defined by the channel's mobile fraction (M_f).

Parameters of channel gating for mutants were indistinguishable from those of wild-type Kv1.3. Confocal microscopy images and co-immunoprecipitation experiments demonstrated that cortactin and Kv1.3 interact in HEK-293 cells. Furthermore, wild-type, delta3 and deltaPDZ mutants have reduced M_f upon stimulation of F-actin polymerization/stabilization by jasplakinolide (unlike delta1, delta2 and deltaSH3 constructs). When cortactin was knocked down by shRNA, exposure to jasplakinolide induced no decrease in M_f of wild-type Kv1.3 channels. These findings point out that cortactin serves as a bridge between Kv1.3 and the actin meshwork via the channel's SH3-binding domain, and can regulate mobility/immobility of Kv1.3 channels. (NIH 2R01CA095286).

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Role of Kv1.3 Potassium Channels in Auditory Function

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Kv1.3 is a low threshold voltage-dependent potassium channel involved in various physiological functions. Within the central nervous system, deletion of Kv1.3 gene from mitral cells of the olfactory bulb dramatically increased the sensitivity of the olfactory system. We have recently shown that Kv1.3 channels are present in presynaptic terminals of the medial nucleus trapezoid body (MNTB) within the auditory brainstem and in bouton-like structures on inner and outer hair cells within the cochlea. Whether Kv1.3 channels contribute to auditory function is, however, unknown. We have therefore used in vivo and in vitro approaches to examine the role of Kv1.3 channels in the peripheral and central auditory system. Auditory brainstem responses (ABR) show that the thresholds of ABR are elevated in 2-4 months old Kv1.3-/- KO mice over those in wild type (WT) mice. Latencies of peaks I, II and IV are prolonged in 4 month old Kv1.3 -/- KO mice. In addition, we have found a desynchronization of ABR waves in Kv1.3-/- KO mice suggesting an alteration of synaptic transmission and changes in spike fidelity within auditory pathways. To further investigate the mechanisms of these alterations in ABR waves in Kv1.3-/- KO mice, we carried out in vitro slice recordings of the high fidelity calyx of Held/MNTB synapse. Our preliminary results from whole cell patch-clamp recordings in young mice (P13-17) show that lack of Kv1.3 channels increases the spike frequency and the spike threshold at presynaptic terminals (Calyx of Held) in response to square pulses of injected currents. Our preliminary data showing that loss of Kv1.3 channels primarily influences the properties of presynaptic terminals and of the ABR waves strongly suggest that Kv1.3 channels are required for normal auditory function.

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Exploring the Effect of Gambierol on the Gating Machinery of Kv3.1 Channels

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Gambierol is a ladder-shaped polyether toxin that acts as a gating modifier to inhibit Kv3.1 channels with nanomolar affinity. Binding determinants for gambierol have been identified at an interface between S5 and S6, located outside the permeation pathway. However, the high gambierol sensitivity of Kv3.1 channels could not be fully transplanted to the insensitive Kv2.1 channel by introducing the S5-S6 determinants. To explore whether also the voltage-sensing domain (VSD) is a determinant for gambierol sensitivity, we exchanged the complete VSD (S1-S4), parts of the VSD (the S1-S3a region and the Sb-S4 paddle), and the electromechanical coupling (L45+S6c) between Kv3.1 and Kv2.1. Our results show that the L45+S6c and the S1-S3a region did not alter the affinity of Kv3.1 channels for gambierol. In contrast, the distal part of the VSD, the S3b-S4 paddle, displayed a 100-fold decrease in affinity compared to WT Kv3.1. Since all VSD chimeras displayed similar biophysical properties and remained sensitive to well-known pore blockers, the loss in gambierol sensitivity in the S3b-S4 paddle chimera is most likely not the result of allosteric effects. Molecular-Dynamics simulations indicated that the S3b-S4 paddle motif resides in proximity of gambierol and that the structure and position of the VSD may regulate the space of the binding site between the pore domain (S5 and S6) and the gating machinery. Hence, our results suggest that the VSD, and especially the S3b-S4 paddle motif, contributes to the structure and/or the accessibility of the binding site for gambierol. (This research was supported by FWO grant G0433.12N to DJS and JT).

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Regulation of K_V1.5 Channel Density in the Rat Atria

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The density of functional Kv1.5 channels underlying I_{Kur}, the main repolarizing current in human atria, is a result of the equilibrium between exocytosis and endocytosis. We have shown that in addition to constitutive exocytosis, Kv1.5 channels can also undergo regulated exocytosis, for instance following changes in the mechanical environment or changes in the cholesterol content of the sarcolemma. Although constitutive and triggered endocytosis have been investigated for Kv1.5, its internalization pathway has not been described. Using high-resolution 3-D deconvolution microscopy, we showed that Kv1.5 channels are associated with clathrin vesicles (CVs) in atrial myocytes. Electron microscopy (EM) showed that CVs are found both at the intercalated disc and at the lateral sarcolemma, aligned along z-bands. Blockade of the clathrin pathway using hypertonic media or SiRNA increased I_{Kur} densityin atrial myocytes and led to Kv1.5 channels accumulating at the sarcolemma, as shown by biotinylation assays and fluorescence recovery after photobleaching (FRAP) experiments. These data support the hypothesis that Kv1.5 channels are internalized *via* the clathrin pathway.

Next, we investigated Kv1.5 channel internalization in a rat model of atrial hemodynamic overload. Despite reduced Kv1.5 protein expression in dilated atria, I_{Kur} density was unchanged, suggesting increased functional Kv1.5 channels at the sarcolemma. Clathrin expression was reduced in dilated atria, and a decreased colocalization between Kv1.5 channels and CVs was observed. However, EM showed no significant difference in internalization activity between sham and dilated atria. Therefore, the reduced clathrin protein synthesis observed in dilated atria is not likely to be responsible for the accumulation of Kv1.5 channels at the sarcolemma. Other mechanisms such as increased recycling and/or membrane stabilization must be investigated to understand how I_{Kur} is maintained in dilated atria.

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Hammond Energy Shifts Reveal Sequence of Conformational Changes in N- and C-Type Inactivation of Kv1.4

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C-type inactivation is sensitive to mutation on extra- and intracellular side of the channel, indicating it may involve conformational changes at both