These findings make apparent the need for more detailed electrophysiological analysis of Kv1.2 and will certainly prove useful to clarify the structure-function relationship of voltage gated potassium channels.

2709-Pos Board B479

Positioning and Guidance of the Voltage Sensor S4 Within the Omega/Gating-Pore in the Shaker K-Channel

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Inwardly leaking omega-currents in resting Shaker K-channels were first found when the long charged residue R362 on S4 was made short, creating a pore in the voltage-sensing domain (Tombola et al., 2005, 2007). We recently demonstrated that in fact a pair of adjacent charged residues on S4 must be short to build a double-gap (Gamal El-Din et al., 2010). These residues located at every third position A359, R362, R365, R368, R371, K374 can be seen as a rail, shorthand notation ''aRRRRK''. Omega-currents were obtained for the double-gap constructs ''asRRRK'', ''RssRRK'' and ''aRssRK'' (short residues lower case). In a mechanistic view, the long residues slide like bolts in a guidance groove which becomes the leaking omega-pore when two occluding bolts are shortened.

Presently, we study the wall and length of this guidance groove. Especially, we checked in the closed state of S4 whether at the outer end of the groove, E283 on S2 opposes A359 on S4, and whether E293, F290 form the inner end and oppose R362 on S4. Firstly, we kept the inner part open (R362S) and studied different mutant pairs at positions 283(S2) and 359(S4). The size of the resulting omega-current clearly corresponded to the cleft width obtained from molecular modeling. Secondly, leaving the outer part open with A359, we demonstrated that the omega-currents now depended on the cleft width between residues at 362(S4) and 293, 290 on S2.

In conclusion, the omega-pore represents a guidance groove for the gating charges of S4. E283 and E293 located at the outer and inner end of the groove determine the length of the membrane voltage drop. This length also guarantees that during gating always at least one residue senses the field in the pore.

2710-Pos Board B480

The Silent K^+ Channel Subunit, $K_V6.4$. Influences the Gating Charge Movement of $K_V2.1$ in a Heterotetrameric Channel Complex

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Voltage-gated K^+ (Kv) channels are tetramers of α-subunits that detect changes in membrane potential (V) by a positively charged (Q) voltage-sensing domain (VSD). Molecular movements of VSDs lead to charge displacement that can be recorded as transient gating currents (I_Q), which subsequently results in channel gating. The silent Kv subunit, Kv6.4, does not form functional homotetramers; however, it can tetramerize with Kv2.1 subunits to form functional Kv2.1/Kv6.4 heterotetramers, with a proposed 3:1 stoichiometry. Previously we showed that Kv6.4 subunits exert a significant (~40 mV) hyperpolarizing shift in the voltage-dependent inactivation of heterotetrameric Kv2.1/Kv6.4 channels, as compared to Kv2.1 homotetramers, without significant effects on activation gating. However, the underlying mechanism remains unclear. To address this we analyzed ionic and I_Q recordings from heterotetrameric Kv2.1/Kv6.4 channels transiently expressed in HEK293A cells. Half-maximal displacement of gating charge ($Q_{1/2}$) for Kv2.1 homotetramers was −26 mV, as determined from the charge-voltage (Q-V) curve. Analysis of the decay time constant of $I_{Q\text{-}ON}$ as a function of voltage resulted in a bell shaped curve with a maximal time constant around the midpoint potential of −20 mV. Co-expressing Kv6.4 with Kv2.1 resulted in earlier charge movement as evident from a ~16 mV hyperpolarizing shift in the Q-V curve. Furthermore, we observed a double bell shaped curve for the decay time constant, with maximal time constants around −20 mV and −70 mV; the latter corresponding to the Kv6.4-induced ~40 mV hyperpolarizing shift in the voltage-dependence of channel inactivation. Therefore, we suggest that this more negatively located ON-gating component presumably reflects the voltage-dependence of the Kv6.4 subunit within the Kv2.1/Kv6.4 heterotetramer, and that the VSD movement of only the Kv6.4 subunit is sufficient to induce closed state channel inactivation.

2711-Pos Board B481

Use of Resonance-Wavelength Grating Optical Biosensors to Detect Channel-Protein Interaction in Slack KNa Channels

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Na+-activated potassium (KNa) channels encoded by the Slack and Slick genes contribute to neuronal adaptation during sustained stimulation and regulate the accuracy of timing of action potentials. Activation of protein kinase C (PKC) increases the amplitude of Slack-B currents and slows their rate of activation. Slack protein is known to interact with a variety of cytoplasmic signaling molecules. Using resonance wavelength grating optical biosensors (the SRU Biosciences BIND system), we have determined that direct pharmacological activation of Slack channels by bithionol produces a sustained decrease in mass distribution close to the plasma membrane, and that phosphorylation of Slack channels mimics this decrease in mass. These results were obtained using transfected HEK293 cells, and confirmed in mouse primary cortical neurons. These changes in mass distribution appear to be specific to the Slack channel because pharmacological activation of the very closely related Slick channel with bithionol does not produce a change in mass distribution. Blocking ion flux through the Slack channel during channel activation does not attenuate this response, indicating that ion flux is not necessary for the change in mass. The very C-terminal domain of Slack has been previously shown necessary for channel-protein interactions, and deletion of this region abolished the observed signal. To determine which proteins or signaling molecules are translocating from the plasma membrane upon channel activation, an RNAi screen against probable channel binding partners was performed, and the Protein Phosphatase 1 (PP1) inhibitor Phactr1 was found to be necessary for this decrease in mass. We hypothesize that activation of Slack by either bithionol or phosphorylation leads to the dissociation of Phactr1 with PP1 from the channel complex, allowing the Slack channel to remain in its phosphorylated and active state.

2712-Pos Board B482

The Potassium Delayed Rectifier Conductance in the Sarcolemma and the Transverse Tubular System Membranes of Mammalian Skeletal Muscle Fibers

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The transverse tubular system (TTS) plays a key role not only in mediating the mechanism of excitation-contraction coupling, but also in determining the electrical properties of mammalian skeletal muscle fibers. We investigated the properties and distribution (between sarcolemma and TTS membranes) of the K delayed rectifier conductance (g_{KV}) by simultaneously recording fluorescence transients and ionic currents (I_{KV}) from FDB muscle fibers stained with the potentiometric indicator di-8-ANEPPS and voltage-clamped using a two-microelectrode configuration. Enzymatically dissociated fibers were mounted on the stage of an inverted fluorescence microscope equipped with a 460-500//500//513-558nm cube. The external solution had (in mM): 150 LiCl, 4 KCl, 20 MOPS, 2 $CaCl_2$, 1$MgCl_2$ and 10 glucose. The Na, ClC-1, Ca and K_{IR} currents were blocked by external TTX (0.001), 9-ACA (0.4), nifedipine (0.02) and Rb (5), respectively. The membrane capacitance was measured after rendering the fibers electrically passive by replacing external Li and K by TEA. We found that I_{KV} records display a delayed onset and decayed markedly during long depolarizing pulses (400ms) due to inactivation and accumulation mechanisms. Furthermore, while di-8-ANNEPS transients recorded from electrically passive fibers displayed quasi-rectangular kinetic properties, transients recorded from control fibers in the presence of I_{KV} were associated with time-dependent attenuations that matched the kinetics of activation and decay of I_{KV} records. Radial cable model simulations were used to evaluate the voltage-dependent kinetic parameters of g_{KV}, to calculate the rate of accumulation of K^+ ions in the lumen of the TTS, and to determine that the relative distribution of this conductance between the surface and TTS membranes is close to equal. This work was supported by NIH grants AR047664, AR041802, and AR054816.

2713-Pos Board B483

Enhancement of Closed-State Inactivation and ER Retention of Kv4.3 Mediated by N-Terminal KIS Domain of Auxiliary KChIP4A

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Auxiliary KChIP4a shares high homology of conserved C-terminal core region with other members of Kv channel-interacting proteins (KChIPs), but exhibits a distinct modulation on Kv4 current expression and gating. It has been shown that the unique N-terminus of KChIP4a functions as K^+ channel inactivation suppressor (KIS) that leads to slow inactivation and current inhibition. However, the mechanism by which the KIS domain causes current reduction remains unknown. In this study, we identified a hydrophobic ER-retention motif within the KIS domain of KChIP4a that suppresses Kv4.3 surface expression using confocal imaging and cell surface biotinylation assay. Further dissection of KIS domain revealed several key residues that cause reduction of Kv4.3 peak current, but do not affect surface expression. Examination of gating properties of Kv4.3 co-expressed with either KChIP4a or its core without the

KIS (KChIP4aΔ2-34) demonstrated that KChIP4a with KIS domain had no significant effect on steady-state activation, but shifted the voltage dependence of steady-state inactivation of Kv4.3 to hyperpolarizing direction by enhancing closed-state inactivation. We have previously demonstrated that KChIP4a can rescue the function of a tetramerization-defect mutant Kv4.3 C110A. The rescued Kv4.3 C110A current is larger than that of WT Kv4.3/KChIP4a co-expression, although C110A surface expression was lower. Upon coexpression, the closed-state inactivation of Kv4.3 C110A mutant was less affected by the KIS domain, as compared with enhanced closed-state inactivation observed in wild-type channel, suggesting a role of T1 domain in mediation of Kv4 closed-state inactivation. Taken together, we propose that N-terminal KIS domain of KChIP4a inhibits Kv4.3 function through dual independent mechanisms by which auxiliary KChIP4a causes Kv4 ER retention and promotes channel closed-state inactivation.

2714-Pos Board B484

Targeted Mutagenesis with a Homology Model Identifies Critical Residues for Arachidonic Acid Inhibition of Kv4 Channels

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Polyunsaturated fatty acids such as arachidonic acid (AA) demonstrate inhibitory modulation of Kv4 potassium channels. Molecular docking approaches using a new Kv4.2 homology model predicted a membrane-embedded binding pocket for AA comprised of the S4-S5 linker on one subunit and several nearby hydrophobic residues within the internal side of S5 and S6 from an adjacent subunit. We tested the hypothesis that modulatory effects of AA on Kv4.2/KChIP channels require access to this site. Targeted mutation of a lysine residue and a nonpolar residue within the S4-S5 linker as well as a nonpolar residue in S3 significantly impaired the effects of AA on K^+ currents in *Xenopus* oocytes. These residues may be important in stabilizing or regulating access to the negatively charged carboxylate moiety on the fatty acid. The structural specificity of this interaction was supported by the lack of disruption of AA effects observed with charge neutralizing mutations at residues located near but not within the predicted binding pocket. Furthermore, we found that the crystal structure of the Kv1.2/2.1 channel chimera lacks an AA docking site with the structural features present in the proposed hydrophobic pocket of Kv4.2 and the chimera was likewise unaffected by AA. We simulated the mutagenic substitutions of critical residues identified in our Kv4.2 model to provide a structural interpretation of the disruption of the AA binding pocket. We conclude that AA inhibits Kv4 channel currents and facilitates inactivation by interacting with a hydrophobic binding pocket in which a lysine residue within the S4-S5 linker is important for AA interaction.

2715-Pos Board B485

Modification of Kv4.2 Channel Complexes by the Diphenylurea Compound NS5806

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NS5806 (N-[3,5-bis(trifluoromethyl)phenyl]-N-[2,4-dibromo-6-(1H-tetrazol-5-yl)phenyl]-urea) can increase the amplitude and slow the decay of currents mediated by voltage-gated potassium (Kv) channels of the Kv4 subfamily if Kv Channel Interacting Proteins (KChIPs) participate in channel complex formation. We studied the effects of NS5806 on the somatodendritic A-type current (Isa) in hippocampal neurons and on the currents mediated by Kv4.2 channels coexpressed with different auxilliary beta-subunits in HEK 293 cells with the whole-cell patch-clamp technique. The effects of NS5806 on the inactivation gating of Kv4.2 mutants were studied under two-electrode voltage-clamp in Xenopus oocytes. Notably, the Isa component in hippocampal neurons was reduced rather than potentiated, but showed a pronounced slowing of inactivation in the presence of NS5806. The peak amplitudes of currents mediated by ternary Kv4.2 channels, coexpressed with different KChIPs and dipeptidyl aminopeptidase-related protein (DPP) 6 were potentiated and their macroscopic inactivation slowed by NS5806. The currents mediated by binary Kv4.2 channels, coexpressed only with DPP6, were suppressed by NS5806 and the effects on macroscopic inactivation were less pronounced. Similar to the Isa component in hippocampal neurons, the midpoint voltage of inactivation of recombinant Kv4.2 channel complexes was shifted negative by NS5806. However, the recovery from inactivation was accelerated in hippocampal neurons and slowed in recombinant channels. Functional analysis of Kv4.2 S4S5 linker and S6 mutants support the notion that NS5806 influences the dynamic coupling between voltage sensor and gate.

Supported by the Deutsche Forschungsgemeinschaft (DFG)

2716-Pos Board B486

Mechanism of Inhibition of Voltage-Gated Potassium Channels by Guanidine Compounds

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Inhibition of presynaptic voltage-gated potassium (Kv) channels by guanidine underlies its use in the therapy for the neuromuscular diseases, myasthenic syndrome of Lambert-Eaton and botulism. The therapeutic use of guanidine is limited, however, due to side effects that accompany its administration. Therefore, the design of guanidine analogs with improved therapeutic indices is desirable. Progress towards this goal is hindered by the lack of knowledge of the mechanism by which these molecules inhibit Kv channels. We examined an array of possible mechanisms of inhibition of the Shaker Kv channel by guanidine, methyl guanidine and *N*,*N*-dimethyl guanidine, including charge screening, disruption of the protein-lipid interfaces, direct interaction with the voltage sensors and pore-binding. Our results demonstrate that guanidines bind within the intracellular pore of the channel, and perturb a hydrophobic subunit interface to stabilize a closed state of the channel. This mechanism provides a foundation for the design of guanidine analogs for the therapeutic intervention of neuromuscular diseases.

2717-Pos Board B487

Sevoflurane: A Potent General Anesthetic Enhancer of Kv Channel Activation

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General anesthetics have been shown to both inhibit and potentiate various families of ion channels. However, the molecular bases of these actions are not understood. Previous work with K-Shaw2 explored structural determinants of inhibition by alkanols and the volatile general anesthetics halothane and isoflurane (Barber et al. Biophys J. 2011, in press). In this study, we report that sevoflurane, an inhaled anesthetic currently used in human general anesthesia, exhibits the singular ability to reversibly potentiate K-Shaw2 currents investigated in Xenopus oocytes under two-electrode voltage-clamp conditions (~80% potentiation at 1 mM). This result is in sharp contrast to the action of multiple other inhaled (halothane, isoflurane, desflurane, chloroform, and 1-alkanols) and injected (propofol) general anesthetics, which are inhibitory. This potentiation occurs over a clinically relevant concentration range (0.05 - 1 mM) in a dose-dependent manner that suggests two binding sites with distinct apparent affinities: a high-affinity site (K1 = 60 μM) and a low affinity site (K2 = 4 mM). Furthermore, a double mutation involving the S6 segment and the S4-S5 linker (A326V/A417V) abolishes the potentiation by sevoflurane. This effect is especially interesting because this double mutation also abolishes the inhibitory action of halothane, suggesting that these inhaled anesthetics share effector sites but influence gating in opposite ways. Semiquantitative kinetic modeling suggests that preferential binding of sevoflurane to resting and activated closed states shifts the channels into a novel gating mode with an enhanced open probability. Additional screening of related voltage-gated K channels at a various doses of sevoflurane revealed only modest potentiation of Kv1.2 no effect on Kv1.3, Kv2.1, Kv3.4 and Kv4.2. This study provides a novel framework for further investigations of the structural basis of general anesthetic action.

2718-Pos Board B488

Activation and Inactivation Kinetics of the Potassium Channels K_V1.3 and K_V1.5 Measured on the CytoPatch™ Instrument and the Manual Patch Clamp: A Comparative Study

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The state of the art method to study ion channels is the patch clamp technology. Since 2003, patch clamp automats offered an efficiently faster analysis method, as it would have been feasible with the manual patch clamp technique. Nevertheless, the advantage of higher throughput in patch clamp automation is accompanied with a lower data quality.
The CytoPatch™ Instrument is a patch clamp automat with a new chip technology, the Cytocentering Technology. With this, the automat is capable to generate a data quality and flexibility comparable to the manual patch clamp technology. To investigate this issue we carried out a comparative study on the activation and inactivation kinetics of the potassium channels K_V1.3 and K_V1.5 measured on the CytoPatch™ Instrument and the manual patch clamp. The data prove that advanced electrophysiological studies can be carried out on the CytoPatch™ Instrument with similar results to the manual patch clamp technique. Furthermore, it goes along with the benefit of automation which is: a higher standardisation - walk away times of several hours - automated data evaluation - no need of a highly-skilled person to run the instrument - higher throughput and lower costs per data point.