3401-Pos Board B448

Differential Effects of the Transient Outward K^+ Current Activator NS5806 in the Canine Left Ventricle

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Objective: The transient outward K^+ current (I_{to}) contributes to repolarization in ventricular muscle. The functional effects of a novel I_{to} activator, NS5806, were determined in canine left ventricular myocytes. **Methods:** Epicardial (epi), midmyocardial (mid) and endocardial (endo) cells were isolated by enzymatic dissociation. Whole cell patch clamp techniques were used to identify I_{to} in the 3 cell types. Oocytes injected with Kv4.3 and KChiP2 were also used to evaluate the effect of NS5806. **Results:** Application of NS5806 (10 μM) to oocytes containing only Kv4.3 *decreased* I_{to} charge by 57% whereas in oocytes containing Kv4.3:KChiP2 (1:1 ratio), NS5806 *increased* I_{to} charge by 45% (at +40 mV). In ventricular myocytes, NS5806 increased the magnitude of I_{to} by 80%, 82%, and 16% in epi, mid, and endo myocytes, respectively (at +40 mV). Similarly, the decay of I_{to} was slowed in the presence of NS5806, resulting in increased total charge of 227%, 192% and 83% compared to control in epi, mid, and endo cells respectively (at +40 mV). Steady-state inactivation of I_{to} was more negative in the presence of NS5806 in all 3 cell types. Patch clamp results suggest the larger increase in I_{to} by NS5806 in epi and mid cells may be due to the presence of a transmural gradient of KChiP2. To confirm, Kv4.3 and KChiP2 mRNA were measured in the 3 cell types. Kv4.3 message was uniform in the 3 cell types, whereas KChiP2 levels were significantly greater in epi and mid cells. **Conclusions:** The activation of I_{to} by NS5806 is dependent on the presence of KChiP2 to increase the magnitude and total charge of I_{to}. Our results suggest a transmural gradient of KChiP2 underlies the transmural gradient of I_{to} in the canine left ventricle.

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The Effect Of The Ito Activator NS5806 On Cloned Kv4.3 Channels Is Dependent On The Accessory Protein KChIP2

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Background: In human and canine ventricles the transient outward current (I_{to}) is mediated by Kv4.3 and various accessory proteins. However, the exact molecular composition constituting I_{to} is still debated. We used the NeuroSearch compound NS5806 known to increase I_{to} in canine ventricular myocytes as a tool to investigate the molecular components comprising I_{to}. **Methods and Results**: The effect of NS5806 was investigated on cloned Kv4.3 channels heterologously expressed together with various β-subunits in *Xenopus laevis* oocytes. NS5806 was found to inhibit Kv4.3 currents with an IC50 of 6.2 μM, whereas it caused a dose-dependent increase in the current carried by Kv4.3 in complex with KChIP2. These results were confirmed in mammalian CHO-K1 cells. The effect of the compound was further tested on Kv4.3/KChIP2 channels in the presence of the accessory proteins KCNE2, KCNE3, DPP6 and DPP10, which were all found to modulate the effect of NS5806. Of the various combinations of subunits investigated, we found that a complex of Kv4.3, KChIP2 and DPP6 resemble the native I_{to} current in canine cardiomyocytes most with regard to the effect of NS5806. The NS5806 effect on Kv4.1 and Kv4.2 was similar to the effect found for Kv4.3 both in presence and absence of KChIP2, whereas the compound had minor effect on Kv1.4 and Kv1.5 channels independent of KChIP2.

Conclusion The effect of the novel I_{to} activator NS5806 on Kv4.3 channels was found to be strongly modulated by the presence of accessory proteins suggesting that NS5806 can be used as a valuable tool compound for addressing the molecular compositions of I_{to} in the heart and of I_A in neuronal tissue in addition to its applicability for testing the physiological role of these currents.

Presynaptic Channels & Release Mechanisms

3403-Pos Board B450

MCell Model of Presynaptic Calcium Dynamics Predicts the Structural Correlates of Short-term Synaptic Plasticity

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Facilitation and depression at synapses can be regulated by the ultra-structure of the synapse. We used the MCell program to perform realistic 3D Monte Carlo simulations of the molecular interactions that regulate transmitter release in a model CA3-CA1 hippocampal synapse. The relatively simple geometry of a hippocampal synapse compared to Calyx of Held allows a quantitative analysis of activity-dependent local calcium profiles at the active zone. Experimentally measurable synaptic variables such as the release probability and the global calcium response were used by the model to predict the structural parameters of the synapse. Tight spatial coupling between voltage gated calcium channels (VGCCs) and the neurotransmitter release complex promoted fast and temporally precise signalling at synapses. This coupling, however, limited the range of facilitation. Since more VGCCs were necessary to maintain release probability for longer coupling distances (up to a few hundred nanometers) it produced an augmented 'global' calcium response in the bouton. The resulting elevated residual calcium could open intracellular calcium stores, cause calcium buffers to saturate and trigger an overall increase in paired pulse ratio. These results were robust to the choice of model parameters and remain valid for a wide variety of neurons. In addition we simulated calcium florescence signals that can be directly compared to measurements with calcium dyes. This model may be extended to explore the molecular mechanisms involved in short-term synaptic plasticity observed in behavioural experiments.

3404-Pos Board B451

Localization of Calcium Channels at the Calyx of Held

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The Calyx of Held is a giant synapse in the auditory pathway. Due to its large size and its geometry it makes an excellent model system for studying the biophysical properties of synaptic transmission. Previous studies have demonstrated that the release rate of synaptic vesicles is governed by the distance between single synaptic vesicles and calcium channels. Therefore knowing the localization of presynaptic calcium channels is essential to understand the coupling of synaptic vesicle release with calcium influx into the calyx. Prior studies have attempted to characterize calcium channel distribution in the Calyx, however results have been inconclusive in providing an accurate picture. In order to conclusively localize calcium channels we are exploring several possibilities to stain calcium channels for fluorescence and electron microscopy studies. The first approach is to express genetically tagged P/Q-type calcium channels in the Calyx utilizing an adenovirus expression system. We have tried an eGFP-tagged P/Q-type calcium channel and found that the eGFP accumulates in the nuclei of the cells indicating cleavage of the tags. The tag also reduced calcium currents twofold compared to untagged channel. Our second approach was to screen several constructs with HA epitope tags inserted into different positions on the P/Q-type calcium channel. We found that extracellular tags frequently impair channel function. However we did obtain constructs which showed current upon expression in HEK cells. Unfortunately accessibility of the tag for the antibody is impaired in neurons. Intracellular tags are accesible in primary neurons and behave similar to untagged channel electrophysiologically, but the staining is difficult to interpret because tagged overexpressed channels accumulate in intracellular compartments. In parallel experiments we have screened several antibodies against P/Q-type calcium channels aiming to find one that gives clean staining for high-resolution microscopy. Funding: LSHM-CT-1005-019055.

3405-Pos Board B452

Computational Study Of The Effect Of Calcium Buffers On The Calcium Current Cooperativity Of Exocytosis

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Synaptic neurotransmitter release is one of the most fundamental processes in physiology, and there is considerable interest in the study of spatio-temporal dynamics of calcium (Ca2+) that triggers exocytosis at a fast chemical synapse. In particular, there his been significant progress in the understanding of the relative coupling of individual Ca2+ channels to the release of a single vesicle. Experimental protocols provide insight into this question by probing the sensitivity of exocytosis to Ca2+ influx. While varying extracellular or intracellular Ca2+ concentration assesses the intrinsic biochemical Ca2+ cooperativity of neurotransmitter release, varying the number of open Ca2+ channels using pharmacological channel block or the tail current protocol probes the cooperativity between individual Ca2+ channels in triggering exocytosis. Despite the wide use of these Ca2+ sensitivity protocols, their interpretation often relies on heuristic arguments, and ignores the potent effect of Ca2+ buffers on

exocytosis. We analyze these experimental protocols, derive simple formulas for special cases, and distinguish carefully between the Ca2+ current cooperativity, defined as the exponent in the relationship between exocytosis rate and the Ca2+ current magnitude, and the underlying Ca2+ channel cooperativity, defined as the average number of channels involved in the release of a single vesicle. Further, we use 3D computational modeling of buffered Ca2+ diffusion to analyze the distinct Ca2+ cooperativity measures, and demonstrate the role of endogenous Ca2+ buffers on such measures. We show that buffers can either increase or decrease the calcium current cooperativity of exocytosis, depending on their concentration and calcium-binding properties, and the distance between channel and vesicle.

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Biophysics of Ion Permeation

3406-Pos Board B453

Toward Controlling The Ion Selectivity By Manipulating Individual Subunits Among Four In A Tetrameric K^+ Channel

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The ion permeation in all the K^+ channels is governed by a selectivity filter that is assembled by backbone carbonyls from four identical conservative sequences, Thr-X-Gly-Tyr-Gly. Varying any part of this sequence for all four subunits often disables the K^+ selectivity. However, it is unclear how the selectivity is altered with an individual subunit among four. Understanding of this mechanism will uncover the contribution of each individual subunit to the overall ion selectivity, i.e. functional stoichiometry. So far this research has been limited due to difficulty in obtaining hetero-tetrameric channel proteins. We are studying this mechanism with a unique model K^+ channel, chlorella virus-encoded Kcv. We have found that the wild-type and tagged Kcv (with an extension of eight asparagines at the N-terminal) can be co-synthesized *in vitro* and self-assembled into various homo- and hetero-tetramers, as visualized through electrophoresis. **Most notably, when purified directly from the SDS gel, each hetero-tetramer exhibited perfect K^+ channel functions in the lipid bilayer** (this is difficult to achieve for other membrane proteins). Using this protein as the background, we obtained all types of hetero-tetramers containing different numbers of the mutant Kcv at the selectivity filter (G65C). The electrophysiology test revealed that the proteins with up to two mutant subunits in the tetramer still retain the K+ selectivity, but the selectivity is disabled for tetramers containing more than two mutant subunits. (*FEBS Letters* 581 (2007) 1027-1034)

3407-Pos Board B454

New Insights Into Selectivity of Potassium Channels Using Small Cation Blockers

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KcsA channel pores are blocked by intracellular Na+ and Li+ ions. We are investigating Na+/Li+ binding locations using electrophysiology, X-ray crystallography, and molecular dynamics simulations. We found that intracellular Li+ blocks KcsA channels with low, voltage-dependent affinity and competes with K+ for the blocking site. Its movement to the blocking site is not coupled with movement of permeant ions in the field. In contrast, Na+ blocks with less affinity and larger voltage dependence. We proposed that both small cations block in the hydrated vestibule with Na+ binding deeper in the pore at a site requiring partial dehydration while Li+ resides lower, remaining fully hydrated. Molecular dynamics calculations indicated low affinity binding for Na+/Li+ in the cavity but also predicted a high affinity binding-site in the S4 site, not ''in-cage'' where K+ ions bind but ''in-plane'' coordinated by Thr75 carbonyl oxygens. In search for all potential Li+ binding-sites we crystallized KcsA in the presence of Li+. Consistent with the MD results, we found three potential binding sites, one of which is in the S4 site of the selectivity filter in the plane of the Thr75 carbonyls. This suggests that Li+ and Na+ may be favored to bind in the S4 site but that they need to overcome a large energy barrier to get there. MD simulations unveil such barriers through free energy calculations involving multiple ion mechanisms for the smaller ions. We are now investigating experimentally the existence of a high-affinity binding-site inside the selectivity filter for both Na+ and Li+.

3408-Pos Board B455

Development of a Drude Polarizable Force Field for Ion-water and Ion-NMA Interactions and Application to Selectivity in Ion Channels

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A challenge in modeling ions in biomolecular systems is the description of interactions involving ions in a heterogeneous environment, where explicit representation of polarization often becomes important. As a first step towards meeting this challenge, a Drude polarizable force field for ion-water and ion-N-methylacetamide (NMA: a model compound for peptide bond) is developed. For the first time, the alkali and halide ion interactions with liquid NMA has been characterized experimentally. By measuring the solubilities in liquid NMA, we derive the solvation free energies of KCl and NaCl in liquid NMA. Good agreements are found for both the structural and thermodynamic properties in the gas phase and in the condensed phase. As an application, the developed polarizable model is used to study ion selectivity in a reduced binding site model of the site S2 in KcsA. The results confirm the previous finding that both the number and type of ligands play an important role in K+ selective ion channels.

3409-Pos Board B456

Cation Blocking Mechanisms of the KcsA Potassium Channel Explored with All-atom Free Energy Simulations

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We have carried out free energy simulations of multiple ion configurations in the KcsA potassium channel to understand experimentally observed Na and Li blocking and offer predictions that are supported by electrophysiological assays and X-ray crystallographic structures. Through free energy perturbation and potential of mean force calculations we find that Na and Li can bind either inside the aqueous cavity of the channel or deep into the S4 site, coordinated by a plane of 4 carbonyl oxygens rather than the usual 8-ligand cage of K. We have found good evidence to support this prediction with the existence of at least two distinct binding sites for Na and Li suggested by the experiments. We demonstrate that a different multiple-ion mechanism is required for Li and Na ion permeation, involving large energetic barriers that are not encountered by K. These studies shed light on how small monovalent cations block the KcsA channel and provide new insight into the selectivity mechanisms of potassium channels.

3410-Pos Board B457

Electrostatic Determinants of Membrane Ion Permeability

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Ion channels facilitate the passage of charged molecules across cell membranes by eliminating energetic costs thought to be associated with dehydration inside a low-dielectric membrane core. However, recent atomistic simulation studies have uncovered a different picture of charge-lipid interactions with reduced barriers due to membrane deformations. Having a correct description of the origins and magnitudes of these energetic barriers is essential to describe ion permeation, as well as to understand processes that involve the interaction of charged peptides or protein domains with membranes. Here we seek energetic decompositions to unveil the mechanisms of assisted or unassisted permeation and explore the roles of membrane electronic polarizability, dipole potential and composition (including charged lipids). We find that while electronic polarizability has some considerable effects on ion solvation free energies in non-polar solvents, as well as solvent interfacial potentials, a polarizable lipid model reveals only small effects on ions in the membrane. We show that the full membrane dipole potential is not seen by ions and explore the role of the membrane electrostatics on ions inside ion channel proteins.

3411-Pos Board B458

Mapping the Common Origins of Ion Selectivity in Biological Molecules

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Numerous biological molecules selectively bind or transport particular ions. In biological systems, the discrimination between sodium and potassium is particularly important. We demonstrate that selectivity of group I ions is dependent