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3802-Pos Board B530

Role of a PH Sensitive Site in Enac Na^+ Self-Inhibition

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The epithelial Na^+ channel (ENaC) is key to the regulation of extracellular fluid volume and blood pressure through its action in the aldosterone-sensitive nephron. Inhibition of ENaC by extracellular Na^+, referred to as Na^+ self-inhibition, likely occurs through an allosteric mechanism of Na^+ binding to low affinity sites in the large extracellular regions of ENaC subunits. The effector binding sites and transduction pathways remain unidentified. A recent comparative model of the ENaC α subunit revealed an acidic cleft, analogous to the acidic region observed in the resolved structure of acid sensing ion channel 1 (ASIC1). We hypothesized that this cleft and analogous clefts in the and subunits host Na^+ effector sites. Mutations of acidic residues in the subunit acidic cleft led us to identify an aspartate on the loop connecting the central palm and β-ball domains that reaches towards the finger domain. We also identified nearby sites on the loop and other structures that affected Na^+ self-inhibition. To determine whether these sites were involved in effector coordination, we measured the ability of mutants to change the effector specificity of Na^+ self-inhibition, which is $Na^+ > Li^+ >> K^+$ for wild type mouse ENaC. Mutation of the palm/-ball loop aspartate and several nearby sites weakened Na^+ inhibition relative to Li^+ and K^+ inhibition. We hypothesized that protonating any carboxylate groups involved in Na^+ coordination would weaken Na^+ binding. Consistent with this, lowering the pH to 4.7 increased ENaC currents and reduced Na^+ self-inhibition. Mutation of the palm/β-ball loop aspartate reversed pH activation of ENaC. Our results suggest that an aspartate in the acidic cleft of the subunit may coordinate Na^+ for Na^+ self-inhibition.

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VDAC-Kinase Electrogenic Complexes as Direct Generators of Mitochondrial Membrane Potentials

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The voltage dependent anion channel (VDAC) is a main metabolic pathway between mitochondria and the cytosol. Although VDAC's electrical characteristics are well known, VDAC is still considered as a constantly open pore in physiological conditions, or as regulated with ''molecular corks'' of various types. On the other hand, one of the unresolved problems, according to Colombini and Mannella (BBA, vol. 1818, 2012), remains the role of VDAC's highly conserved voltage gating properties. Earlier, we have proposed several possible mechanisms of generation of the mitochondrial outer membrane potential (OMP). According to one of them, the OMP might result from the application in part of the inner membrane potential (IMP) to the outer membrane through the intermembrane contact sites ANT (adenine nucleotide translocator)-VDAC, or AND-VDAC-HK (hexokinase). Even VDAC-HK electrogenic complex has been considered to function as a direct generator of the OMP, using the Gibbs free energy of the HK reaction. Here, we further developed this concept showing with a simple computational model that the creatine kinase (CK) reaction might also contribute to the OMP generation applying its Gibbs free energy to the ANT-CK-VDAC contact sites, or to the CK-VDAC complex, similarly to that suggested earlier for the ANT-VDAC-HK or VDAC-HK complexes. The OMP might influence the distribution of adenine nucleotides and calcium between the intermembrane space and the cytosol, thus influencing mitochondrial metabolic state and permeability transition. One of the results of the developed concept is a new, non-Mitchell mechanism of the IMP generation under hypoxic/anoxic conditions. Thus, VDAC seems to function as an electrical metabolic interface playing fundamental role in various physiological processes by controlling the metabolic state of mitochondria and cell resistance to death.

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Characterization of the Intrinsic Voltage-Dependent Gating Mechanism of CALHM Ion Channels

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Human CALHM1, which plays important roles in cortical neuron excitability and taste perception, belongs to a new family of evolutionarily conserved ion channels that are regulated by voltage and extracellular Ca^{2+} (Ca^{2+}_o) [1,2]. There is a single hCALHM1 homolog in *C. elegans,* CLHM-1 [3]. Although hCALHM1 and CLHM-1 have little sequence identity (16%), these ion channels exhibit similar biophysical properties when expressed in *Xenopus* oocytes and are functionally interchangeable when expressed *in vivo* [3].Since neither hCALHM1 nor CLHM-1 contain known Ca^{2+}-binding sequences or canonical voltage sensing domains, we are seeking to identify residues responsible for the intrinsic voltage-dependent gating mechanism for CALHM channels. To understand the Ca^{2+}_o and voltage-dependent gating mechanisms utilized by these ion channels, we determined activation and deactivation kinetics as well as apparent conductance-voltage (G-V) relations for CLHM-1 and hCALHM1 in divalent-free and Ca^{2+} containing solutions. While both channels were regulated by Ca^{2+}_o and voltage, there were significant differences in the kinetics and voltage dependencies in the absence of Ca^{2+}_o. These results suggest that the intrinsic gating of these channels is significantly different. To define the channel region(s) required for intrinsic voltage-dependent gating, we have examined the gating kinetics and apparent G-V relationships of CLHM-1 / hCALHM1 chimeric proteins. A chimeric protein consisting of CLHM-1 with the second extracellular loop from hCALHM1 forms a functional ion channel. Like hCALHM1, it is difficult to close this chimeric channel in the absence of Ca^{2+}_o at hyperpolarized voltages. This result suggests that the second extracellular loop may play a role in intrinsic voltage-dependent gating of CALHM ion channels.

[1] Ma et al., PNAS (2012).
[2] Taruno et al., Nature (2013).
[3] Tanis et al., J. Neurosci. (2013).

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Navigating the Terra Incognita of Male Fertility: Sperm Ion Channels and their Function

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Mammalian spermatozoa are unable to fertilize an oocyte immediately after their deposit in the female genital tract. To gain competence to fertilize, they must undergo a gradual maturation process termed capacitation that is accompanied by: 1) sperm intracellular alkalinization, evoked by proton extrusion through proton channels; 2) elevation of the intracellular $[Ca^{2+}]$, evoked by calcium influx through calcium ion channels, and 3) membrane hyperpolarization that is evoked by K^+ efflux. Whereas the proton and calcium channels of human sperm are identified as Hv1 and CatSper, the identity of the principal potassium channel of human sperm (hKSper) was unknown. Potassium channels are indispensable for normal sperm physiology as they regulate cell membrane potential and cell motility. Recently, a pH-sensitive sperm K^+ channel, encoded by the Slo3 gene was shown to be regulated by intracellular alkalinization and essential for male fertility in mice. It has been assumed, but never proven, that the K^+ channel of human sperm has a similar molecular identity. Here we present full electrophysiological and pharmacological characterization of hKSper in both mature and developing human spermatozoa, and report that the molecular identity of hKSper is different from that of mouse KSper. In human sperm, the KSper current is conveyed by the big conductance, calcium-activated potassium channel (Slo1) which differs from mouse Slo3 in both its regulation and functional relationship with other sperm ion channels.

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Initial Functional and Structural Analysis of Plant Mechanosensitive Channel MSL10

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MSL10 is the first mechanosensitive channel from *Arabidopsis thaliana* to be electrophysiologically characterized. MSL10 is a member of the MscS family of which there are 10 in Arabidopsis. Though MSL10 is topologically different from known bacterial MS channels, its most C-terminal TM helix shows moderate homology with *E. coli* MscS's pore-lining domain. MSL10 mechanosensitivity has been shown both in exogenous (*Xenopus* oocytes) and endogenous systems. However, nothing was known about the structure and organization of any eukaryotic MscS homolog. Using mutational analysis, electrophysiology, and *in planta* assays, we identify residues important for MSL10 function, concentrating on its cytoplasmic N-terminus, 6 cysteine residues located both on cytoplasmic and extracellular sides and in TM helices, and the putative pore-lining domain. Recently we have identified MSL10's N-terminus as a multiple phosphorylation site, which suggests its importance for channel regulation. A set of mutants mimicking either phosphorylated or non-phosphorylated forms of the MSL10 protein was tested by patch-clamp when expressed in *Xenopus* oocytes and simultaneously studied *in planta*. According to our model, phosphorylation of the N-terminus alone does not affect channel function. Instead, it mediates an unknown step in cell death signaling. MD simulations have suggested an impact of phosphorylation on the structure of MSL10's cytoplasmic N-terminus. We have also demonstrated that cysteine pairs on the cytoplasmic (C205, C283) and on the extracellular (C95, C581) side of the channel do not form bonds critical for channel assembly and gating. And finally, mutations introduced into MSL10's putative pore-lining domain (V549S, F553S; F553L and F563L) allowed us to identify residues that affect channel gating and stability. Therefore, this work has revealed several important structure-function relations for the newly characterized family of MS channels in plants.