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Cx26 (hCx26) channels seems to represent the channel with open gates. This structure, and molecular dynamics studies based on it, reveal that charged residues (D46, E47, R75, R184) at the extracellular entrance of the aqueous pore - a region thought to be involved in gating rearrangements - form an electrostatic network. We explored the role of these salt bridge interactions in gating using mutagenesis, kinetic analysis and chemical modifications. Substitution of neutral residues for D46 or E47, which would eliminate their participation in salt bridges, accelerate deactivation kinetics and moderately increase the apparent affinity of Ca^{2+} to induce channel closing. These data support a role of these residues in stabilization of the open state. In addition, when D46 is substituted by a cysteine (D46C), modification by MTSES to add a negative charge increases holding and tail currents. This suggests that a negative charge at this position is involved in stabilizing open hemichannels. In wild-type channels, following depolarizing pulses to 0 mV, peak tail currents increase as a function of pulse duration, reaching maximum with pulses of 40 sec. Strikingly, E47A/Q mutations showed peak tail currents that saturate more rapidly, at 15 sec, suggesting that this position also plays a key role in hemichannel activation. Thus far, our data suggest that intra- and inter-subunit electrostatic networks at the extracellular entrance of the hCx26 pore play critical roles in hemichannel gating reactions. Support: R01GM099490.

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Extracellular Divalent Cations Regulation of Cx26 and Cx30 Hemichannels

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Due to the large size and modest selectivity of the aqueous pore, exacerbated opening of connexin hemichannels leads to loss of electrochemical gradients and of small cytoplasmic metabolites, causing cell death. Control of hemichannel opening is indispensable, and is achieved by extracellular divalent concentrations, which drastically reduces hemichannel activity. Here, we explore the differences between extracellular Ca^{2+} and Mg^{2+} regulation in two relatives connexin, hCx26 and hCx30. Our standard protocol for assessment connexin hemichannel activation and deactivation with the two electro-voltage clamp technique is to examine the peak tail currents and their relaxation kinetics following a depolarizing pulse from −80 mV to 0 mV. using this protocol, the peak tail currents increase with reduction of external divalents. We estimate the extracellular Ca^{2+} and Mg^{2+} apparent affinity for hCx26 hemichannels at values of 0.33 mM and 1.8 mM, respectively. hCx30 hemichannels showed slightly higher extracellular Ca^{2+} and Mg^{2+} apparent affinity with values of 0.17 mM and 1.0 mM, respectively. At physiological Ca^{2+} concentration (1.0 - 1.8 mM), both hCx26 and hCx30 hemichannels reach $\leq$ 15% of the maximal response, but at corresponding Mg^{2+} concentrations they reach $\geq$ 50%. In addition, deactivation time constant at the tail currents are accelerated as a function of Ca^{2+} concentrations in hCx26 and hCx30 hemichannels; however, only high extracellular Mg^{2+} concentrations ($>$ 2.0 mM) are capable to accelerate deactivation kinetics in both connexin types. The holding currents at steady state are significantly increased at physiological extracellular Mg^{2+} concentrations (1.0 - 1.2 mM) in both hCx26 and hCx30 suggesting an increase in open hemichannels even at negative potentials. Our data support that, under physiological ionic conditions, Ca^{2+}, but not Mg^{2+} plays a major role stabilizing and facilitating closing of Cx26 and Cx30 hemichannels. Support: R01GM099490

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The Effect of Arachidonic Acid on Junctional Conductance and Gating of Connexin 36 Gap Junction Channels and their Modulation by N-Alkanols

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Arachidonic acid (AA) is one of major components of membrane phospholipids and is actively involved in the regulation of junctional conductance (g_j). *N*-alkanols are well known inhibitors of gap junction (GJ) channels and have been shown to reduce neurological tremors. In this study, we examined function of Cx36 GJ channels, which are expressed in neurons and β-cells of pancreas, under control conditions and application of factors modulating concentration of AA in the plasma membrane. We found that in *HeLa* cells expressing Cx36-EGFP, short carbon chain alkanols (SCCAs), such as pentanol, hexanol and heptanol, increased g_j by ~3-fold. Conversely, long carbon chain alkanols (LCCAs), such as octanol, nonanol and decanol, uncoupled cells fully. We demonstrate that under control conditions only ~0.003 of Cx36 GJ channels assembled in junctional plaques are functional, and this fraction increases by SCCAs and fatty acid free bovine serum albumin (BSA). BSA increased g_j by 1.6-fold with EC_{50} of 2.3 M, while its modified form 1,2-cyclohexanedione (BSA-CHD), which does not bind AA, was ineffective. Voltage sensitive gating of Cx36 GJs was reduced by SCCA and BSA that explain in part their g_j-enhancing effect. The inhibition of Cx36 GJ channels by AA can be rescued by BSA but not by BSA-CHD. MAFP and thapsigargin, inhibitor and activator of AA synthesis via phospholipase A_2, increased and reduced g_j, respectively. We assume that endogenous AA is one of key factors leading to low functional efficacy of Cx36 GJ channels under control conditions. Furthermore, we suggest that g_j-enhancing effect of BSA and MAFP may be related with reduction of AA levels, while SCCAs limit AA's accessibility to its binding site on Cx36.

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Ion Channels Formed by SARS Coronavirus Envelope Protein: Lipid Regulation of Conductance and Selectivity

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Coronaviruses (CoV) are pathogens that cause common colds, bronchiolitis and acute respiratory distress syndrome. In fact, their relevance increased when the causative agent of the severe acute respiratory syndrome (SARS) was identified as a CoV. CoV E protein is a small transmembrane protein of between 76-109 amino acids in length that modulates coronavirus morphogenesis, tropism and virulence [1].

We have reported that E protein conductance and ion selectivity were controlled by the lipid composition of the membrane [2]. These results indicated that, most likely, lipid molecules assembled with the peptide oligomers to form the channel.

Here we provide additional evidences of the functional involvement of lipids in the channel structure. The influence of lipid molecules on E protein channel transport properties was investigated focusing on the salt concentration dependence of the E protein conductance and the pH dependence of the channel ion selectivity.

The channel conductance in neutral bilayers increased with the electrolyte concentration whereas in charged bilayers it is approximately proportional to the square root of salt concentration, which reveals an electrostatic contribution from the lipid charge. In regard to pH dependence of ion selectivity, in uncharged bilayers the titration curve shows a single transition that corresponds to E protein residue titration, whereas in charged bilayers a second transition is observed, which presumably corresponds to lipid groups titration.

These results support the previous hypothesis that the lipids are functionally involved in E protein ion channel activity, forming a protein-lipid pore, a novel concept for CoV E protein ion channel entity.

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[2] Verdiá-Báguena C., et al. 2012. Virology. 432: 485-494.

3250-Pos Board B405

Electrophysiology of Concatameric Pannexin 1 Channels Reveals the Stoichiometry of C-Terminal Autoinhibition

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Pannexin 1 (PANX1) is a non-selective ion channel that mediates the uptake of cyanine dyes and release of nucleotides and other metabolites. PANX1 activation and ATP release/dye uptake are regulated by diverse stimuli, including physicochemical factors (e.g., stretch, K^+ ions) and signaling by various G protein-coupled and ionotropic receptors. We recently described a unique regulatory mechanism for the release of ATP from apoptotic cells: PANX1 is activated by caspase cleavage of a C-terminal autoinhibitory domain. Current evidence suggests that PANX1 channels are hexameric, and cleavage-resistant subunits can interfere with caspase-dependent activation in a dominant-negative fashion. To explore the subunit stoichiometry required for C-terminal autoinhibition, we engineered concatameric PANX1 constructs