averaging indicates that the Cx26WT N-terminus is very flexible. The fitting of a recent X-ray crystallographic structure of Cx26 into the Cx26M34A and Cx26M34Adel2-7 2D crystal maps reveals radial shifts of the transmembrane helices toward outside of the channel. This movement of the six monomers within each of the two hemichannels may reflect a difference between an open state (3D crystals) and a closed state (2D crystals), but also reflects that in the 2D crystals, the channels are surrounded by two lipid bilayers. In addition, the channels in the 2D crystals show complex structural features at cytoplasmic side distinct from the 3D crystal structure. Thus, flexibility of inter subunit interactions and rearrangement of an N-terminus to form a ''plug'' create a closed channel for the M34A mutant.

493-Pos

Oligomeric State of Purified Wild-Type and Deafness-Associated Mutants Solubilized in Decylmaltoside

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Gap-junction channels are formed by head-to-head docking of two hemichannels, which are connexin hexamers. Gap-junction channels and hemichannels are permeable to large hydrophilic solutes (up to M_r ~1,000, depending on the isoform). Mutations of Cx26 are the most frequent cause of genetic deafness. In these studies, we expressed wild-type Cx26 with a C-terminal His tag to aid in affinity purification. Purified Cx26 solubilized in decylmaltoside was subjected to analytical gel filtration to determine its oligomeric state. We found that purified Cx26 consists of a number of oligomeric states, including monomers, hexamers and dodecamers. Wild-type Cx26 hexamers reconstituted in liposomes formed functional hemichannels, as demonstrated by sucrose-permeability assays. Purification in the presence of the reducing agent TCEP yielded more hexamers and less aggregates, whereas further incubation with TCEP resulted in an increased fraction of Cx26 monomers. We have shown that the dominant mutant R75W is incapable of forming gap-junction channels, but forms hemichannels with altered voltage dependence of the open probability, without changes in single-channel conductance. The R75A mutant does not form functional hemichannels. The fraction of Cx26 that is present as hexamers was similar for the wild type Cx26 and the R75W mutant, but it was reduced significantly in the case of the R75A mutant. The latter also displayed a significant increase in aggregation. These results suggest that the single R75A mutation decreases Cx26 hemichannel stability, which associates with the absence of functional hemichannel formation in frog oocytes injected with Cx26 R75A cRNA. This work was supported in part by NIH grants R01GM79629 and R21DC007150, American Heart Association Grant-in-Aid 0755002Y, and a grant from the Center for Membrane Protein Research of TTUHSC.

494-Pos

Deafness Mutation A88S Induces Cell Death Due to Impairment of the Slow Gating of HCX26 Hemichannel

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Connexin hemichannels can be gated by both extracellular Ca^{2+} and membrane voltage. The latter gating has two components, fast and slow, which transit the channels from open to subconductance or fully closed state, respectively. Here, we show that a non-syndromic sensorineural deafness mutation of human Cx26A88S, impairs the slow gating responsible for the depolarization induced opening and hyperpolarization induced closing of these hemichannels, while leaving the fast gating response of hemichannels and all gating of gap junction channels unaffected. Thus, under hyperpolarizing voltages comparable to cochlear supporting cells, Cx26A88S hemichannels have a persistent current that WT hemichannels do not have. As a consequence, expression of Cx26A88S in *Xenopus* oocytes induces a dramatic increase in cell lysis compared to those expressing WT hemichannels, an effect that is blocked by incubation in 2 mM extracellular [Ca^{2+}]. This is the first implication of a role for the slow voltage gating of hemichannels in the etiology of deafness. Intriguingly, the deafness mutation at A88 in Cx26 is actually the wild-type residue in Cx50, which forms hemichannels that show a persistent residual current in low extracellular Ca^{2+}. A Cx50S89A mutant confers depolarization activation, and a slow gating response that completely closes the channel, similar to the properties of wt Cx26. Thus, we have identified a residue critical to slow gating of hemichannels, but not fast gating or gating of gap junctions, that is conserved in the connexin family, and is important for different tissue functions. The location of this residue in the recently published crystal structure of Cx26 indicates that substitution of A88 with serine could result in a hydrogen bond with R143 on the third transmembrane segment, potentially limiting a critical movement required for slow gating of hemichannels.

495-Pos

Hemichannels in Thymocytes: Participation in Apoptotic Processes

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The body of evidence suggesting the existence of functional hemichannels has been increasing in different organs such us: heart, brain, and ear. However, the presence of functional hemichannels in immune system cells remains an open question. Previously, we demonstrated that thymocytes express Cx30.3 and Cx43. Nevertheless, they do not form functional gap junction channels between them or with thymic epithelial cells. For this reason, we decided investigated if the connexin found in thymocytes could function as hemichannel. We observed a generation of an ionic current and dye uptake when thymocytes are submitted to low extracellular calcium (permeabilization assay and flow cytometry) and a positive pipette potential [Vp (patch clamp technique)]. We demonstrated that hydrocortisone could modulate the thymocytes hemichannels open probability, even in the presence of 1 mM of extracellular calcium. Since hydrocortisone is a potent apoptotic inducer we tested if caspases could be implicated on the hemichannel open. We found that the caspase-9 blocker inhibited the hemichannels open. We showed the presence of functional hemichannel in thymocytes and we suggested that the opening of hemichannel could be involved in the thymocyte apoptosis.

496-Pos

Vascular Gap-Junction Cx37 Uncoupling By Tumor Necrosis Factor is Dependent on ZO-1 Expression

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Regulation of gap junctional intercellular communication plays a very important role in many physiological and pathophysiological processes. Despite significant knowledge of the role of endothelial cells during inflammation, the function of specific endothelial connexins during inflammation is not well understood. Our hypothesis is that tumor necrosis factor (TNF) will decrease gap junction dependent cell-to-cell communication of vascular connexin by disturbing connexin-cytoskeleton interactions.

Transformed HeLa cells expressing vascular connexin 37 (gift from Dr. Klaus Willecke) were used in these experiments. HeLa cells were treated with TNF (20 ng/ml) for up to 2 h. In dye-transfer experiments, Alexa Fluor-480 (HeLaCx37) was injected into one cell for 10 s and the number of labeled cells counted after 10 m. Cell lysates were prepared and ultracentrifuged. ZO-1, N-cadherin, actin, and Cx37 were detected by Western blot. Cx37 was also immunoprecipitated (IP) overnight and precipitated.

After 1 hour, TNF treatment resulted in near total loss of dye-coupling in HeLaCx37 ($p<0.02$, n=16) and remained constant up to 2 hours. siRNA-mediated knockdown of ZO-1 restored dye coupling. TNF caused a significant increase in detergent solubility of Cx37. ZO-1 was co-IP with Cx37 only after TNF treatment, suggesting that TNF induces a ZO-1 and Cx37 interaction. Actin was co-IP with Cx37 but TNF did not affect this association. N-cadherin was not co-IP with Cx37. Immunofluorescence double labeling for Cx37/ZO-1 and Cx37/actin confirm the co-IP experiments.

TNF reduces gap junction coupling of Cx37 when expressed alone in epithelial cells. The loss of Cx37 function maybe due to the loss of detergent resistance, suggesting dissociation of Cx37 plaque. TNF mediates Cx37 interaction with ZO-1 but not actin. N-cadherin does not interact with Cx37. TNF may affect Cx37/ZO-1 interaction resulting in reduced dye coupling.

497-Pos

Calcium-Calmodulin Regulation of Connexin43 Involves a Cytoplasmic Loop Domain

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Connexin43 (Cx43) is widely expressed throughout the mammalian body and is the predominant gap junction protein in the ventricular myocardium. Cx43 was recently reported to contain a calmodulin (CaM) binding site on its cytoplasmic loop (CL) domain near its third transmembrane domain (Zhou et al., JBC 282: 35005-17, 2007). Intracellular calcium (Ca_i)-dependent regulation of cardiac and Cx43 gap junctions has also been reported, but the function of this putative CaM-binding site has never been directly examined. In dual whole cell patch clamp experiments, murine neuro2a (N2a)-Cx43 cell gap junctional conductance (g_j) declined by 95% within 10 min (n=3) during bath perfusion with 1 μM ionomycin + 1.8 mM external Ca^{2+} (Ca_o). Cx43 g_j declined by only