20% in nominally zero Ca_o (n=5). This Ca^{2+}-dependent uncoupling was demonstrated to be CaM-dependent by acute (10-15 min) pretreatment with 2 μM calmidazolium, wherein Cx43 g_j declined by < 10% within 10 min (n=4). To directly test for the involvement of the Cx43 amino acid residue #136-158 domain in this Ca^{2+}/CaM-dependent gap junction uncoupling process, 1 μM peptides were added to both whole cell patch pipettes and the 1 μM ionomycin/1.8 mM Ca_o perfusion experiments were repeated. The Cx43 #136-158 sequence mimetic peptide (K_d(CaM) = 860 nM) effectively prevented the Cx43 g_j decline (< 3%, n=4) whereas a scrambled sequence peptide control failed to prevent the Ca^{2+}-induced rundown of Cx43 g_j (<90%, n=3). These data unequivocally demonstrate that influx of external Ca^{2+} induces closure of Cx43 gap junctions in a CaM-dependent process involving the Cx43 residue #136-158 CL domain. This process has significant implications for the modulation of cardiac g_j by Ca_i and the ''healing-over'' of infarcted myocardium. Supported by NIH grants GM62999 & EY-05684 to JJY and HL-042220 to RDV.

498-Pos

Cam Interaction and Binding Mode Study with Peptide from Intracellular Loop of Cx50

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Connexin 50 (Cx50) is a member of the α family of gap junction proteins expressed in the lens of the eye where it has been shown to be essential for normal lens development. We have identified calmodulin (CaM) binding sites in the intracellular loop near to the 3rd transmembrane region of Connexin43 (Cx43) and Connexin44 (Cx44) which belong to the same α connexin family as Cx50. Sequence alignment of the candidate CaM binding regions of Cx43 and Cx44, with Cx50 identified a region encompassing residues 141-166 of Cx50 with a high predicted affinity for CaM. A peptide $Cx50_{141-166}$ was synthesized to study the interaction of CaM with this domain of Cx50. Biophysical results indicate that in the presence of Ca^{2+}, $Cx50_{141-166}$ binds with high affinity (0.14 μM) to CaM, as monitored by NMR spectroscopy and measured by the fluorescence intensity change of IAEDANS that was covalently attached to the C-terminal Cys of CaM. Electrophysiological data support the hypothesis that elevated intracellular Ca^{2+} concentration inhibits Cx50 gap junctions because omission of Ca^{2+} from the 1 μM ionomycin bath saline prevented the 95% decline in junctional conductance (g_j) observed in the presence of 1.8 mM $CaCl_2$. This is likely CaM-mediated, because inclusion of a CaM inhibitor also prevented this Ca^{2+}-dependent inhibition of Cx50 gap junctions. The involvement of the Cx50 CaM binding domain in this Ca^{2+}/CaM-dependent regulation was further demonstrated by inclusion ofthe $Cx50_{141-166}$ peptide in both whole cell patch pipettes, which effectively prevented the usual decrease in Cx50 g_j. These results demonstrate that the binding of Ca^{2+} -CaM to the intracellular loop of Cx50 is critical for mediating the Ca^{2+}-dependent inhibition of Cx50 gap junctions in the lens of the eye.

499-Pos

Gating Modulation of Connexin45 Gap Junction Channels By Intracellular pH

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Intracellular pH (pH_i) changes considerably during pathological conditions such as ischemia or epilepsy. Changes of pH_i affect the way cells communicate through gap junction (GJ) channels, and therefore disturb normal tissue function. To study pH_i-dependent modulation of GJ channels, we used HeLa cells expressing connexin45 (Cx45) or its fusion form with EGFP. The latter along with electrophysiological data allowed us to estimate the proportion of functional channels (N_F) within a junctional plaque (JP). We examined how junctional conductance (g_j) depends on pH_i and how pH_i affects voltage-gating properties of Cx45 homotypic and Cx45/Cx43-EGFP heterotypic GJs. Even at pH_i= ~8, where the probability of the channels being fully open approximates 1, g_j was maximal but only 5 % of the channels were functional. Changes in pH_i from ~7.2 to ~8 increased g_j ~1.8-fold in homotypic Cx45 GJs; g_j-pH_i dependence was sigmoidal with pK_a = ~7. We used a stochastic four-state model of contingent gating to fit experimental g_j-V_j dependence, which allowed us to define parameters characterizing voltage-gating sensitivity ($V_0$and A) and N_F. We found that alkalization increases g_j mainly by increasing V_0, i.e., voltage at which open and closed states of hemichannel are at equilibrium. On the other hand, uncoupling by acidification was due to a decrease of both V_0 and N_F. In both cases, the constant A, characterizing the steepness of g_j changes over V_j remained stable. These results agree with data obtained from heterotypic Cx45/Cx43-EGFP GJs in which pK_a= ~6.7, i.e., in between pK_as of Cx43-EGFP and Cx45 homotypic GJs. In summary, pH_i modulates V_j-gating that largely explains observed pH-dependent changes of cell-cell coupling.

500-Pos

Single Channel Connexin43 Plaque Formation

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Connexin43 (Cx43) is one of the most ubiquitous gap junction proteins in the human body and plays an essential role in cell-to-cell communication for a variety of organs and organ systems. Gap junction hemichannels are composed of six, often identical subunits, which can range from 26 kD to 60 kD, which assemble into water tight ion conduits which bridge the extracellular space between opposing cells and allow transfer of electrical impulses and small solutes up to 1 kD. Single hemichannels have been shown to remain functional in a cell membrane even when unopposed and have been linked to propagation of intercellular calcium waves, release of NAD+ and ATP, neuronal signaling, and the activation of many different kinase cascades. Here, we explore the electrophysiological properties of single Cx43 and Cx43eGFP hemichannels and their interactions during plaque formation in a planar lipid membrane (BLM). The average conductance of a Cx43 channel was found to be 753 ± 31 pS (n = 30) for a 500 mM KCl buffer. Cx43eGFP exhibited an average conductance of 783 ± 53 pS (n = 30). Unlike in-vivo patch clamp experiments, Cx43 was purified and isolated from other membrane constituents, producing a system capable of probing both connexon electrophysiology and the roles of several well known gap junction blockers, namely: lanthanum, carbenoxalone and lindane. We also use single channel BLM to examine the critical number of hemichannels required for plaque formation and the emergent electrical properties therein.

501-Pos

New Classes of Gap Junction Channel Blockers for Cx43 and Cx50

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In many tissues gap junction channels as well as hemichannels play important roles in intercellular electrical and biochemical coupling, cell synchronization, differentiation, growth and metabolic coordination. Therefore they have been proposed as potential new targets for the treatment of diseases such as epilepsy, cardiac arrhythmia and cancer. However, highly specific and potent pharmacological tools to further study their physiological as well as pathophysiological role are missing. The existing gap junction channel modulators are either of low potency, cross-react with other ion channels or exhibit no subtype specificity. To identify potent and selective gap junction blockers we screened a small library of compounds containing ion channel modulating pharmacophores. We identified five small molecule chemotypes including quinolines and triarylmethanes (TRAMs) that inhibited intercellular coupling via Cx43 or Cx50 in the lower micromolar range.

The triarylmethane derivatives, e.g. T66 (*N*-[(2-chlorophenyl)(diphenyl) methyl]-*N*-(1,3-thiazol-2yl)amine) (IC_{50} 3 μM), blocked Cx50 currents with IC_{50} values in the range of 1-10 μM while having only small or no effects on other gap junction channel subtypes such as Cx32, Cx36 and Cx46. The quinoline derivative SB002 (4-(4-phenoxybutoxy)quinoline) inhibited Cx50 (IC_{50} 3 μM) as well as Cx43 (IC_{50} 8.3 μM). We currently are exploring the structure-activity relationship (SAR) to increase potency and for the quinoline derivatives to shift the subtype selectivity profile towards Cx43.

We propose quinolines as well as triarylmethanes as new pharmacological tool compounds to further elucidate the physiological roles of Cx43 and Cx50 and to study their contribution to disease pathogenesis.

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502-Pos

Heteromultimeric Gap-Junction Channel Permeance: Directional Fluxes Simulated Using a Brownian Dynamics Model

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Permeance for distinct connexin channels has been hard to predict. Heterotypic connexin combinations yield preferential directional fluxes of fluorescent molecules with small influence from particle charge, suggesting regulation through differences in pore's shape. We have simulated particles' movement across a 3D geometric pore's representation from X-ray crystallography following a Brownian Dynamics Model (BDM). A central prolate represents the pore's vestibule; a cone and a cylinder represents the pore's mouths for Cell 1 (C_1) and Cell 2 (C_2) respectively. Lucifer yellow molecules ($e=0$ or $-2e^-$, Stokes radius of $4.9\mathring{A}$) were represented as spheres. Particle-channel charge interactions were simulated placing a charged ring near the channel's mouth. BDM described closely particle behavior where the displacement vector (dx) was calculated using particles' diffusion matrix, net force and a random vector from