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DISSERTATION

"Gating regulation of Connexin26 hemichannels by cytosolic interdomain interactions"

by

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ABSTRACT

Connexin hemichannels are hexameric ion channels that are trafficked to the plasma membrane preceding the formation of gap junction channels (GJCs) between adjacent cells. Typically, these hemichannels are closed in under normal resting potentials, which is achieved by the negative potential of the cell and physiological extracellular Ca^{2+} concentrations that significantly reduce open channel probability and the channels. Structural and functional data indicate that the Ca^{2+} sensing domain is located towards the extracellular side of the hemichannel pore. When Ca^{2+} ions are bound to the pore, they form an electrostatic ring that restricts, but does not prevent, access of ions and molecules to the cytoplasm. This suggests that the conformational changes accompanying hemichannel closing by extracellular Ca^{2+} concentrations must involve a gate in another region of the pore. In addition, structural studies indicate that the N-terminal (NT) domain in connexin hemichannels folds into the pore, where it plays important roles in permeability and gating. These findings have led other groups to propose the NT domain as a physical gate of connexin channels. Supporting this idea, a group of human mutations within the NT domain of connexin26 (Cx26) cause deafness and skin disorders, including Keratitis-Ichthyosis-Deafness (KID) syndrome. These mutants have been shown to increase hemichannel activity. Our central hypothesis is that *the N-terminus is responsible for the closed state of the channel, acting as a gate, and interaction between the N-terminus and the cytoplasmic loop stabilizes the open state*. We studied human mutations located throughout the NT and CL to gain insights about how they affect gating mechanisms. We observed robust changes in gating properties with the NT KID mutant, N14K, and explored the molecular basis of how N14K promotes gain in function. In macroscopic and single channel recordings, we observed that the N14K mutant stabilizes the open conformation of hemichannels, shifts calcium sensitivity, voltage sensitivity, and slows deactivation time constants. Molecular Dynamics (MD) simulations showed that the N14K mutation disrupted wild-type (WT) pairwise interactions between N14 and K15 in the NT with residues (H100 and E101, respectively), which are at the transition between transmembrane segment 2 and the cytoplasmic loop (TM2/CL) of the adjacent subunit. Double mutant cycle analysis of these pairwise interactions supports thermodynamic coupling between NT and the TM2/CL in the WT hemichannels that is disrupted in the N14K mutant hemichannels. Correlation analysis obtained from MD simulations suggest that the changes in the distribution of interactions due to the N14K mutation are not confined to these two pairs (N14-H100 and/or K15-E101) but extends to essentially all correlated pairwise movement of NT residues with residues at the NT - TM2/CL interface. This suggests that NT-CL/TM2 interactions facilitate Cx26 hemichannel closure. Taken together, our work suggests that the NT domain plays critical roles in gating and is highly coupled to the Ca^{2+} sensing domain on the extracellular side of the channel. In addition, we studied differences in the conformational state of the NT domain when the channel was opened or closed by introducing a cysteine at the second residue of the NT domain. We found that coordination to Cd^{2+} ions or disulfide bond formation between the NT domains blocked hemichannel currents. The kinetics of the hemichannel current decay in response to TbHO2 or Cd^{2+} was greatly accelerated at higher extracellular Ca^{2+} concentrations. Our data strongly suggest that: 1) The NT domain of adjacent subunits come in close proximity when the channel is closed by extracellular Ca^{2+}, and 2) there is an allosteric coupling between the Ca^{2+} sensing domain at the extracellular side and the NT domain at the intracellular side of the pore.