Effect of mutations on common gating in human ClC-1 chloride channels

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Monomers of dimeric ClC channels have their own protopore, independently fast-gated, perhaps, by the carboxyl side chain of E232. Both fast gates are then simultaneously regulated by slower, common gating, with an unknown structural basis. Dominant negative effects on common gating in mutant/WT heterodimers, however, suggests intersubunit allostery. We propose that an interaction pathway between fast gates could constitute this “common gate”, closing both fast gates, because mutations that affect it are clustered at the dimer interface. In patch-clamped HEK cells, these mutants have common gate open probabilities (P_o^c) shifted tens of mV in the depolarising direction and are blocked by Zn^{2+} much faster than WT channels. By contrast, human ClC-1 mutants C277S and C278S display little common gating or block by Zn^{2+}, implicating these residues in Zn^{2+} coordination. Alternatively, with decreased common gating, a specific closed substate of the common gate might be less available for stabilisation by Zn^{2+}. Candidate residues for a possible interaction pathway can be found in the helix-helix interaction motifs, GxxxG(S), in helices G and H with helix H at the dimer interface and helix G lying deep to H and closer to the putative protopore. We have mutated helix G residues, A272, G270, G274 and G276 and their close helix H neighbours, S289, V292 and T293. Common gating in V292L was almost eliminated, while for the other mutants, and especially A272V and S289L, P_o^c were shifted to more depolarised potentials. As anticipated from its suppression of common gating, but unexpectedly since C277 and C278 remained intact, V292L was not subject to block by Zn^{2+}. Of the other mutants, all were blocked, with A272V most rapidly affected. Thus some quite subtle mutations indicate residues likely to be important in the interaction pathway underlying common gating. Interestingly, regarding our hypothesis, mutation of the putative fast gate (E232Q) also suppressed common gating and Zn^{2+} block.