Functional characterisation of ten myotonia-associated mutations of the hClC-1 chloride channel

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Myotonia congenita (MC) is a rare muscle stiffness disease of humans, and some other animals, associated with mutations in the gene, *CLCN1*. When correctly expressed and trafficked, its protein product, the wild type (WT) skeletal muscle chloride channel, ClC-1, passes chloride currents that buffer the depolarising influence of t-tubular potassium accumulation on membrane potential. This prevents involuntary after-discharges following tetanic muscle contractions. At least 120 different human ClC-1 (hClC-1) mutations are now known, although details of less than 80 have so far been published (Colding-Jørgensen, 2005) and less than half of these have been analysed to determine the specific reason for their failure to function or to be expressed (Pusch, 2002; Grunnet *et al.*, 2003; Simpson *et al.*, 2004). We have begun to investigate the remainder by heterologous expression and whole-cell patch-clamping in cultured HEK293 cells. Plasmid hClC-1 cDNA constructs were prepared by site-directed mutagenesis to mimic the naturally-occurring mutants and we used these to transfect our HEK cells. We also co-expressed some severely truncated mutants with complimentary C-tail peptide fragments to obtain functional reconstitution and unambiguous evidence that expression of the mutant had been achieved. Typically, after 24-48 hours had elapsed, cells were tested for evidence of voltage-gated chloride currents and/or hClC-1 protein expression. Using these methods, ten previously uncharacterised, myotonia-associated, hClC-1 mutations (S70L, G190R, C271R, G276D, W303R, G305E, E717X, S728L, Q807X and fs872X) were studied. No chloride currents could be obtained from cells transfected with G276D, G305E, E717X or Q807X. Robust functional rescue of truncations E717X and Q807X could, however, be achieved when either was co-expressed with C-tail fragment, G721-L988, indicating strong expression, correct trafficking to the plasma membrane and a potential for future therapy of these and similar truncations caused by premature stop codons. Interestingly, the open probabilities of both the fast gate (P_o^f) and common gate (P_o^c) of W303R and fs872X were shifted by tens of mV in the depolarising direction, sufficient to keep these channels almost closed within the activation range of WT channels. Minimum values of P_o^c for fs872X and C271R were reduced by ~70%, or more, at negative potentials and $V_{1/2}$ for C271R was shifted in excess of +150mV. These characteristics are typical of hClC-1 mutants that have been associated with the dominant form of myotonia congenita as these mutant subunits have a dominant negative effect on WT subunits when paired in ClC-1 channel dimers. From one compound heterozygous patient (S70L/S728L), neither mutant was significantly different from WT. Some of these myotonia-associated genetic variations may prove to be benign hClC-1 polymorphisms, the actual cause of the myotonia lying, for example, in the SCN4A sodium channel. Unexpectedly, however, chloride currents in cells transfected with the G190R mutant (associated with dominant MC) were also similar to WT, despite G190 being central to the chloride-binding motif, GSGIPE, and almost totally conserved across the CLC family of proteins from prokaryotes to humans. It is apparent that an absence of chloride currents in the case of some missense mutants and truncations could explain their association with recessive MC and that the association of other mutants with dominant MC could be accounted for by their strikingly altered voltage dependence of gating. More evidence will be required, however, to reveal any solid linkage between several of the mutants and either form of MC.

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