The sensitivity of myotonia congenita hClC-1 mutants to 9-AC provides insight into the structure-function relationship and pharmacological modulation of hClC-1 channels

C. Altamura[1], P. Imbrici[1], A. Toscano[2], R. Mantegazza[3], M. Lo Monaco[4], J-F. Desaphy[1],D. Conte Camerino[1]

[1]Dept. of Pharmacy - Drug Sciences, University of Bari 'Aldo Moro', Bari, Italy
[2]Dept. of Neurosciences, University of Messina, Messina, Italy
[3]Neurology IV - Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS Neurological Institute Carlo Besta, Milan, Italy
[4]Institute of Neurology, School of Medicine, Catholic University of the Sacred Heart, Rome, Italy

The voltage-dependent ClC-1 chloride channel is mainly expressed in skeletal muscle where it supports the large resting chloride conductance that normally stabilizes muscle fibre membrane potential. Loss-of-function mutations in the ClC-1 channels are associated to myotonia congenita (MC), an inherited skeletal muscle channelopathy characterized by muscle stiffness after a voluntary movement and various levels of transient weakness (1). The reduced chloride conductance due to ClC-1 mutations predisposes the sarcolemma to the hyperexcitability that hampers muscle relaxation after contraction (myotonia). To date, no drug exists able to directly activate ClC-1 channel and revert MC induced ClC-1 loss-of-function or improve its trafficking. Conversely, compounds able to block ClC-1 have proved useful pharmacological tools to develop animal models of myotonia or to investigate ClC-1 structure-function relationship. 9-anthracene-carboxylic acid (9-AC) is one of the most potent organic compound inhibiting selectively heterologously expressed ClC-1 channels but not ClC-0 and ClC-2. This compound binds to a partially hydrophobic pocket close to the chloride binding site that is accessible from the cytoplasm, and its action is voltage-dependent, reducing currents mostly at negative voltages (2).
We recently identified five novel ClC-1 mutations causing MC in Italian families associated to a Thomsen or Becker clinical phenotype. The G190S and L198P located in helix D, the G270V located in helix G, the F484L situated in helix N and the L628P in the CBS1 (3,4,5). We have previously shown that these pore mutations greatly right shift the voltage dependence of activation thus reducing the amount of chloride current at physiological membrane potentials causing myotonia. Conversely, the C-terminal mutation shows a biophysical profile similar to WT. Interestingly, these pore mutations reside close to the pore residue S537 taking part to the putative binding pocket for 9-AC.
By whole-cell patch clamp, here we tested the sensitivity to 9-AC 300mM, applied from the outside, of MC mutant channels expressed in tsA201 cells in order to gain insight into the structure-function relationship of ClC-1 channels, and to obtain valuable information for the rational design of much-needed therapeutic agents.
Our experiments show that F484L channels are insensitive to 9-AC block suggesting that the aromatic moiety of the phenylalanine 484 could be involved in hydrophobic interactions required for drug-channel binding. In addition, 9-AC blocks G270V and L198P channels only slightly compared to WT channels. Interestingly, in the presence of 9-AC the chloride currents of G270V increase slowly upon depolarization suggesting that 9-AC block is relieved at inside-positive voltages that tend to open G270V channels. Conversely, the L628P mutation located in the CBS1 domain, thus outside the putative 9-AC binding site, does not affect 9-AC block compared to WT channels.
In conclusion here we show that spontaneous ClC-1 mutations associated to MC located in the channel pore not only affect ClC-1 function but also ClC-1 pharmacology. Importantly, using these natural MC mutants we identified additional residues, located in the channel pore in helices D and G, that are relevant for 9-AC binding. Mutations of these residues might hamper 9-AC reaching its blocking binding sites through conformational changes and/or interfere with this inhibitor. Docking studies are in progress. It is expected that such pharmacological and structural information would contribute to the development of highly desirable compounds able to positively modulate ClC-1 mutant channels. Supported by Health Ministry (GR-2009-1580433) and Telethon-Italy (GGP14096).

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