Molecular determinants for 9-AC and NFA binding to ClC-1 chloride channelsand possible chaperone activity on myotonic ClC-1 mutants.

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Myotonia congenita (MC) is an inherited muscle disease characterized by impaired muscle relaxation after voluntary contraction, resulting in muscle stiffness. It is caused by loss-of-function mutations in the skeletal muscle chloride channel ClC-1, affecting its biophysical properties or membrane expression. Currently no direct activators of ClC-1 are known.
So, the ideal drug would be one able to directly open ClC-1 channel in case of gating-defective mutants, or one capable to increase surface expression in case of trafficking-defective MC mutants. A strategy to develop compounds able to act in this direction can exploit small molecules, highly selective, reversible ClC-1 blockers, that are currently available such as 9-anthracene carboxylic acid (9-AC) and niflumic acid (NFA).
Here, we first provide an in-depth characterization of 9-AC binding pocket in ClC-1 to increase knowledge regarding ClC-1 structure-function relationship using electrophysiological and docking studies. Second, we tested the ability of 9-AC and NFA to act as a pharmacological chaperone on one trafficking-defective MC mutant. To this purpose, WT and MC mutant ClC-1 channels were expressed in HEK293 cells and whole-cell currents were recorded with patch-clamp, before and after external application or incubation with 9-AC and NFA.
To study 9-AC binding site, we took advantage of recently characterized ClC-1 mutations causing MC in Italian families (1,2,3) and tested their sensitivity to 300 μM 9-AC. Four pore mutations G190S, L198P, G270V and F484L reside close to the putative 9-AC binding pocket and induce a reduction of chloride current by right shifting the voltage dependence of activation. F484L channels completely loose sensitivity to 9-AC, suggesting that the aromatic moiety of F484 could be involved in hydrophobic interactions required for drug-channel interaction. In addition, block of G190S, L198P and G270V channels by 9-AC is reduced compared to WT. Interestingly, in the presence of 9-AC, G270V currents increase slowly upon depolarization suggesting that 9-AC block is relieved at inside-positive voltages that open G270V channels.
Docking studies suggest that 9-AC binds in the pore region of ClC-1 and envisage the critical role of the K231 in 9-AC binding. For this reason, we test the sensitivity of 9-AC on K231A ClC-1 mutant channel. 9-AC slighly blocks the K231A mutant channel, confirming docking simulation.
To verify if 9-AC and NFA interact with ClC-1 channels in the same binding pocket, we study the effect of NFA on K231A mutant channels.
Our results show that NFA blocks K231A mutant channels, as much as WT.
We further investigated whether 9-AC and NFA acts as a pharmacological chaperone for the A531V mutant channels, which show a reduction of channel surface expression due to increased protein degradation (1). Incubation of transfected cells for 24 hours with 30 μM 9-AC and 50 μM NFA increase both instantaneous and steady-state A531V chloride currents compared with control conditions, maintaining voltage-dependence similar to WT.
In conclusion, we show that K231 and F484, located in the pore region, are high-impact residues for 9-AC binding in ClC-1. Additional residues including G190, L198 and G270 may be relevant for 9-AC to reach its binding site and block ClC-1 channels, directly or through conformational changes.
The different sensitivity of K231A for 9-AC and NFA may suggest that these compounds interact with different residues of the channel.
Moreover, this study provides a proof of concept that small ClC-1 ligands, such as NFA and 9-AC, can function as pharmacological chaperones of trafficking-defective ClC-1 mutants and may prove useful to restore sarcolemma chloride conductance in myotonic patients.

(1) (Desaphy et al., 2013) Exp Neurol 248:530-540
(2) (Imbrici et al., 2015) J Physiol 593(18):4181-99
(3) (Portaro et al., 2015) Neuromol Med 17(3):285-96