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IGF-1 effect, supporting the involvement of a serine-threonine phosphatase in IGF-1 activity (De Luca et al., *Br. J. Pharmacol.* 1998). Angiotensin receptor antagonists and other tools are in use to gain further insight in the mechanisms involved in inflammation-sensitive ClC-1 impairment in muscular dystrophy (Telethon-Italy GGP05130).

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Evaluation of the Effects of Statin and Fibrate Treatment on Rat Skeletal Muscle: Biophysic, Genetic and Proteomic Studies

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Resting chloride conductance (gCl) sustained by the ClC-1 chloride channel have a crucial role in muscle physiology by maintaining the sarcolemma electrical stability. Its reduction can produce myotonia-like symptoms. ClC-1 channel has been shown to be a target of therapeutic molecules such as statins and fibrates. We previously demonstrated that lipophilic statins and fibrates affect skeletal muscle function by reducing resting chloride conductance (gCl) (Pierno et al, *Br J Pharmacol* 149:909, 2006). Here we studied the time course changes of fluvastatin (20 mg/kg) and fenofibrate (60 mg/kg) effects on Extensor Digitorum Longus (EDL) muscle gCl measured by two-microelectrode current clamp method. The gCl decreased in a time-dependent manner, being significantly lower after 1 week (2464 ± 66 $\mu S/cm^2$, n=46 and 2510 ± 53 $\mu S/cm^2$, n=50 in fluvastatin and fenofibrate treated rats, with respect to 2706 ± 83 $\mu S/cm^2$, n=27 of control). To investigate the causes of gCl reduction we analyzed the ClC-1 gene expression by real-time quantitative PCR. The results showed a marked decrease in ClC-1 mRNA expression in both fluvastatin and fenofibrate chronically treated animals which contributes to gCl reduction. To study the involvement of other proteins essential for muscle function we analysed the proteomic map of EDL muscle from rat treated with fluvastatin (20mg/kg), atorvastatin (10mg/kg), fenofibrate (60mg/kg) and with combined fluvastatin (5mg/kg) plus fenofibrate (30mg/kg) by two-dimensional gel electrophoresis (Gelfi et al, *J Proteome Res* 5:1344, 2006). Fluorescent stained proteomic map showing ~500 spots were obtained and 40, 74, 60 and 76 differently expressed proteins were found in the above mentioned treated groups, with respect to control. The identification of each spot will allow to identify the protein targets of the myopathic process.

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Anomalous Mole Fraction Effect in ClC-2 Chloride Channel Pore

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The Cl^- pathway of E. Coli ClC H^+/Cl^- exchanger has three binding sites that can be occupied by Cl^-, however $SeCN^-$ (an anion similar to SCN^-) occupies only one site. This pathway serves as a model for pore structure of ClC channels whose gating is coupled to permeation. Thus ClC channels may gate differently in the presence of SCN^-. We analyzed the relationship between gating and pore properties by performing SCN^- mole-fraction experiments in ClC-2 channels expressed in HEK cells. Internal and external solutions with different SCN^- fractions were prepared mixing $[Cl^-] + [SCN^-] = 140$ mM. Replacing 100% Cl^- with SCN^- on both sides of the membrane shifted to the left the voltage (V_m) vs channel P_0 curve by ~-18 mV without changing the slope. In contrast, the protopore gate P_P vs V_m curve was shallower and shifted rightward (+5 mV). Extracellular SCN^- mole-fractions produced negative shifts on reversal potential (E_R) values which are not described by GHK equation with a constant P_{SCN}/P_{Cl} ratio. The P_{SCN}/P_{Cl} ratios increased from 1.4 to 2.5 when the mole fraction increased, suggesting extracellular SCN^- enters the pore better than Cl^-. In addition, the slope of P_0 vs V_m curve was steeper and the mid-point voltage ($V_{0.5}$) did not change. Intracellular SCN^- mole fractions produced nonlinear negative shifts on E_R suggesting that from this side Cl^- enters the pore better than SCN^-. The slope of the P_o vs V_m curve was shallower and $V_{0.5}$ and slope conductance vs mole fraction displayed the classical anomalous behaviour. Interestingly, the protopore gate located in the Cl^- pathway also displays the same behaviour. Our data show that the ClC-2 channel has indeed a multi-ion pore and that SCN^- enters this pore preferably from the extracellular side.

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Pharmacological Characterization of GaTx2, a Peptide Inhibitor of ClC-2 Chloride Channels

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ClC-2 chloride channels are voltage-gated ion channels that are expressed in neuronal and epithelial cells where they are critical mediators for the passive diffusion of Cl^- across the plasma membrane. Although ClC-2 is nearly ubiquitously expressed in mammalian cells, many details regarding channel biophysics and the physiological role that ClC-2 channels play remain undefined. We recently isolated the first peptide toxin active against ClC channels: Georgia anion toxin 2 (GaTx2), a 3.2 kDa peptide composed of 29 residues with three disulfide bonds. Here, we describe the basic pharmacological features of the inhibitory activity of GaTx2 against ClC-2, including affinity, mechanism of inhibition, and specificity. Using two-electrode voltage-clamp, we created a dose-response curve for inhibition of ClC-2 by GaTx2 at $V_M = -100$ mV, and calculated a K_D of 22 $\pm$ 10 pM. This value was very similar to the value obtained from dose-response curves created from multi-channel patches, which gave a K_D of 12 $\pm$ 5 pM. Additionally, from TEVC recordings we measured $k_{on} = 43 \times 10^6$ $M^{-1}s^{-1}$, and $k_{off} = 0.0034$ s^{-1}, which is consistent with rate constants for other peptide inhibitors. Single channel recording showed that the latency to first opening is increased nearly 8 fold in the presence of 20 pM GaTx2. Also, outside-out macropatches revealed that GaTx2 is unable to inhibit open ClC-2 channels; thus, this toxin may act as a gating modifier. Finally, we found that GaTx2 is specific for ClC-2, being unable to inhibit other ClC channels or transporters, other major classes of Cl^- channels, or voltage-dependent K^+ channels. This high affinity, highly specific inhibitor of ClC-2 will provide an excellent tool for studies designed to understand the function and regulation of this channel, and will help define its physiological role(s).

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Slow Gating in ClC Chloride Channels: Normal Mode Analysis

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All-atom NMA is used to explore possible mechanisms for slow gating in ClC Cl^- channels. As the ‘‘double-barreled’’ architecture is well established throughout the ClC family, both channels and transporters (Miller, 2006. *Nature.* **440**:484), we use the high-resolution (2.5 Å) X-ray structure of an *E. coli* ClC transporter (pdb entry 1OTS) as a template, describe it with the CHARMM22 force field and carry out standard all-mode NMA. The slowest, intrinsic motions encoded in the structure are determined by protein shape. Perturbing the system in either direction along the 7th all-atom NM leads to slow relative swinging of the subunits, perpendicular to the membrane plane. The in-plane swivel axis lies at the subunit interface, near the protein's center. The intracellular interfacial domain is the region most affected. Here the two halves of the protein oscillate, separating and then nearly touching. The R and A helices execute large scale swaying, alternately increasing and decreasing their cytoplasmic ends' separations, motion in agreement with FRET experiments (Bykova et al., 2006. *Nat. Struct. Biol.* **13**:1115). The ion-occupied intracellular pores behave as almost rigid units. As the subunits separate, the intracellular pore tilt relative to the membrane plane changes notably. In contrast, the extracellular portion of the subunit interface is significantly less affected, although small interfacial structural changes are clearly observable. Those extracellular regions structurally affected by the subunits' slow sway are localized near the extracellular Cl^- pathways. As the subunits separate, these regions compress, possibly shutting the extracellular pores. As they approach, the extracellular regions near the Cl^- conduction pathways relax, possibly opening them.

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***Ab Initio* Calculations Of Structural Rearrangements and Energetic of Glutamate^{148} Site Chain of the Ec-ClC H^+/Cl^- Exchanger**

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ClC H^+/Cl^- exchangers are homodimers with three Cl^--binding sites within each monomer. Previous studies suggest that transport cycles are triggered by protonation of the lateral chain of a glutamate residue (E148) located in the middle of the Cl pathway. In this work, we performed *ab initio* density functional theory calculations using the ultra soft pseudopotential approach in order to gain insight about microscopic movements induced by H and/or Cl during transport activity. For these computations the 16 amino-acids that line the Cl pathway were spatially aligned according to the X-ray structure. We found that the orientation of the unprotonated E148 carboxy group was influenced when Cl was in close proximity. Attaching a single H to this COO^- group displaced the lateral chain (with respect to the unprotonated structure) towards the extracellular side and led to the formation of a sizable hole in the entryway. When Cl was placed at 2Å from the protonated COO^- group (COOH) (in both intra- and extra-cellular positions) the conformational