whereas the reactivity of T338C was much less sensitive to the presence of the blocker. Finally we used a homology model of CFTR to estimate the relative positions of 337 and 338 in relation to the anion-selective pore. The model predicts that, whereas 338 is positioned such that it would be expected to directly line the pore, 337 could be partially occluded by other helices. Further, a molecular dynamics simulation suggests that movement of the transmembrane helices would significantly alter occlusion of F337C and have much less effect on T338C. These results demonstrate that by combining a quantitative analysis of the reactivity of substituted cysteines with an atomic-scale CFTR model we can begin to reveal the architecture of the CFTR anion-selective pore. Supported by NIH, CF Foundation and American Lung Association.

2418-Pos Board B388

On the Mechanism of CFTR Inhibition by CFTRinh-172

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CFTR is inhibited with high potency and selectivity by thiazolidinone, CFTRinh-172. It was reported that CFTRinh-172 decreased the Po by increasing the mean closed time (τ_c) without changing the mean open time (τ_o). These findings lead to the conclusion that CFTRinh-172 acts on the closed state. However, our data show that CFTRinh-172 not only increases τ_c, but also decreases τ_o by ~40% ([CFTRinh-172] = 5 μM). For wild type (WT)-CFTR, the dose response relationship of CFTRinh-172 shows a Ki of 1.433 ± 0.235 μM. Interestingly, G551D-CFTR, which manifests a τ_c ~100 fold longer than that of WT-CFTR, demonstrates a similar degree of inhibition as WT-CFTR. In contrast, manipulating the channel open time dramatically affects the degree of inhibition. For example, 1 μM CFTRinh-172 causes 49% inhibition of the ATP-induced current, but the same concentration of CFTRinh-172 inhibits 82% of the current elicited by N^6-phenylethyl-ATP, a high affinity ATP analogue that opens CFTR with a longer open time. The Ki of CFTRinh-172 for WT-CFTR locked open by ATP and PPi was estimated to be 5-10 nM. This drastic shift of the dose-response relationship is also seen with E1371S CFTR, a hydrolysis-deficient mutant. Due to impaired hydrolysis at NBD2, the current relaxation of E1371S mutant upon ATP withdrawal is very slow, 0.012 ± 0.002 s^{-1}. In the presence of 5 μM CFTRinh-172, the rate of current relaxation of E1371S increases to 0.289 ± 0.096 s^{-1}. This result indicates that the inhibitor acts after the channel is open. The rate of the recovery from inhibition is 0.0227 ± 0.0038 s^{-1} for WT-CFTR, but 0.0036 ± 0.0020 s^{-1} for WT-CFTR locked open by PPi, and 0.0022 ± 0.0004 s^{-1} for E1371S. We conclude that the open state of the channel is favorable for CFTRinh-172 action and that the longer the open state, the higher the affinity for CFTRinh-172.

2419-Pos Board B389

Functional Study of CBS Domain Interaction during Common Gating of CLC-0 Chloride Channel

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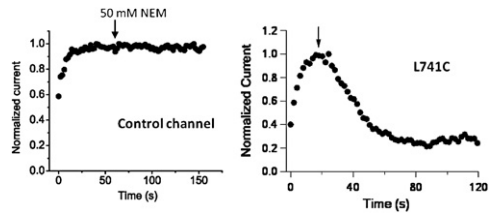
A pair of tightly interacting cystathionine β-synthetase (CBS) domains serves important regulatory functions in various protein families. Two CBS domains (CBS1 and CBS2) exist in the C-terminal of all CLC channels and appear to mediate most of the cytoplasmic inter-subunit interactions. Previous study in our lab indicates that common gating of CLC-0 is associated with a large conformational change in the C-terminal. How interaction between CBS domains affects common gating remains elusive.

Based on three recently reported crystal structures of the C-terminal of CLC channels (CLC-0, CLC-5 and CLC-Ka), we identified a set of residues that likely contribute to CBS interaction. Mutations at most of these positions affected the gating kinetics as well as the equilibrium of common gating. Preliminary data indicate that cysteine mutations at these positions can be modified by thiol-reactive reagents. Furthermore, the kinetics of common gating changed after cysteine modification. These results indicate that CBS domains do play an important role in common gating.

The figure shows the time course of NEM modification of L741C (right); the mutation was made in the control channel (left) background that lacked native reactive cysteines.



2420-Pos Board B390

Deuterium Isotope Effects On Fast Gating Of The Chloride Channel Clc-0

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Gating of the *Torpedo* Cl^- channel ClC-0 is modulated by intracellular and extracellular pH, but the mechanism responsible for this regulation has remained so far elusive. Using inside-out patch clamp measurements we studied the dependence of the fast gate on pH_{int} and $[Cl^-]_{int}$. Only the closing rate, but not the opening rate showed a strong dependence on these intracellular factors. Using mutagenesis we excluded several candidate residues as mediators of the pH_{int} dependence. We propose a model in which a proton generated by the dissociation of an intrapore water molecule protonates E166 leading to channel opening. Deuterium isotope effects confirm that proton transfer is rate limiting for gate opening and that channel closure depends mostly on $[OH^-]$. The model is in natural agreement with the finding that only the closing rate constant, but not the opening rate constant, depends on pH_{int} and $[Cl^-]_{int}$.

2421-Pos Board B391

R-helix Movement during Common Gating Affects Cl Binding in the CLC-0 Channel Pore

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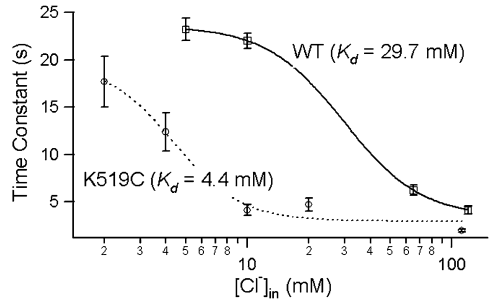
Common (slow) gating of CLC-0 generates long silent periods in single-channel recordings and contributes significantly to regulation of Cl- permeation. Our previous study suggests that movement of the pore-forming R-helix is directly coupled to common gating. We now report that R-helix movement appears to directly interfere with Cl- binding in the pore. Binding of Cl- to the pore facilitates common gate opening, while removing Cl- slows down common gate opening. Mutations in R-helix that strongly affect common gating also appreciably shift the Cl- dependence of channel open rate, apparently by altering the binding affinity of Cl- in the pore. In this way, the common gating mechanism of CLC-0 is reminiscent of the fast gating, which also involves the control of chloride binding to the pore.



2422-Pos Board B392

Skeletal Muscle Chloride Channel, a Biophysical Sensor of Dystrophic Progression in Mdx Mouse, is a Potential Target of Pro-inflammatory Mediators

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A decrease in resting chloride channel conductance (gCl) characterizes myofibers of dystrophic mdx mouse in relation to both spontaneous degeneration, as in diaphragm, or exercise-induced damage as in fast-twitch EDL muscle (De Luca et al., *J. Pharmacol. Exp. Ther.* 2003). The molecular mechanism underlying gCl impairment might involve change in ClC-1 channel expression/turnover and/or function. Considering the role of inflammation in dystrophic damage, we tested if pro-inflammatory mediators may have ClC-1 channel as a target, through phosphorylating/dephosphorylating pathways. Two microelectrodes current clamp recordings were used to measure resting gCl in EDL and diaphragm muscle fibers from adult wild-type (wt) and mdx mice. In line with previous evidences, the *in vitro* application of phorbol dibutyrate (50 microM) reduced gCl of wt EDL myofibers from 2610 ± 240 microS/cm^2 (n=30) to 1265 ± 180 microS/cm^2 (n=15). The application of TNF-alpha (1-30 ng/ml), a cytokine highly expressed in dystrophic muscle, to wt EDL fibers reduced gCl in a concentration-dependent manner with a maximal significant 20% decrease at 10-30 ng/ml. Angiotensin-II (10-100nM), possibly involved in muscle degeneration and oxidative stress, produced a concentration-dependent decrease of gCl in wt EDL myofibers, with a 40 % decrease at 100 nM. The PKC-inhibitor chelerythrine (1 microM) contrasted the effect of either phorbol ester, TNF-alpha or angiotensin-II. The application of 3.3 nM IGF-1 to diaphragm and EDL muscle fibers from exercised mdx mice, significantly counteracted the 40% impairment of gCl. Okadaic acid (0.25 microM) fully prevented