but this static picture alone cannot depict the protein movements that must occur during ion transport. To address this issue, we have utilized fluorine NMR to monitor substrate-induced conformational changes in ClC-ec1. We show that substrate-driven conformational change is not constrained to the Cl^- permeation pathway alone, and that the ClC-ec1 subunit interface participates in protein movement. Furthermore, removal of the protein's H^+ transport ability does not eliminate the H^+-dependent protein movement observed in the intact antiporter. Finally, we observe that a ClC-ec1 ''channel-like'' mutant is not subject to the same substrate-dependent conformational changes that occur in the ClC-ec1 transporters. Together, these results provide new insight into conformational change in ClC-ec1 and lay an essential foundation for future studies on ClC-ec1 protein dynamics.

1668-Pos

Cardioprotective Role For the CLC-3 Chloride Channel in the *mdx* Mouse Model of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is the most common human X-linked disease affecting 1/3,500 male births. DMD patients suffer from severe, progressive muscle wasting with clinical symptoms first detected between 2 to 5 years of age. As the disease progresses patients are confined to a wheelchair in their early teens and die in their early twenties from cardiopulmonary failure. There is currently no effective treatment or cure for DMD. DMD patients and *mdx* mice (the mouse model for DMD) have mutations in the dystrophin gene that result in an absence of the dystrophin protein. Dystrophin is a 427 kDa protein located under the sarcolemma on the inner cytoplasmic membrane of skeletal and cardiac muscle cells and provides structural and functional integrity to muscle. Although therapies have been developed that target skeletal muscle disease, increasing evidence suggests correcting the cardiomyopathy is critical to the survival of DMD patients. As the cardiomyopathy progresses, the hearts of DMD patients and *mdx* mice exhibit arrhythmias, conduction abnormalities and left ventricular dilation. Recent studies have shown that the chloride ion channel ClC-3, a candidate protein responsible for volume-regulated chloride channels in heart, plays a critical cardioprotective role against the development of hypertrophy and failure (*J Mol. Cell. Cardiol.* 2009 Jul 15. [Epub ahead of print]).Using transgenic mice including heart specific ClC-3 knockout *mdx* mice, echocardiography and electrophysiology, we analyzed the role of ClC-3 in modulating cardiac disease progression in dystrophic mice. Our preliminary results indicate ClC-3 may be a major modifier of cardiac disease progression in *mdx* mice and targeting ClC-3 expression or function may provide a novel therapeutic approach for the treatment of dilated cardiomyopathy in DMD (supported by 3P20RR015581 from the National Center for Research Resources).

1669-Pos

Blocking Kinetics of CFTR Channel by Aromatic Carboxylic Acid Positional Isomers Characterised using a Novel Amplitude Distribution Analysis Method

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To investigate the pore structure of the cystic fibrosis transmembrane conductance regulator (CFTR) channel, we performed a systematic pore probing on CFTR channel pore with a series of small aromatic carboxylic acids, including their positional isomers, e.g., 9-anthracene carboxylic acid (9-AC) and 1-anthracene carboxylic acid (1-AC).

Small compounds presumably interacting the channel protein with a few points are sensitive to structural changes of the binding site. However such low affinity blockers show fast - intermediate blocking kinetics which give us the overall affinity, but not on- and off- rates separately. To overcome this problem, we developed an iterative simulation method to estimate the on- and off- rate constants in the 9-AC or 1-AC block from the single channel amplitude distribution.

The newly developed Amplitude Distribution Analysis (ADA) program first generated a single-channel current according to the given kinetic scheme and added a Gaussian noise to the currents for mimicking the background noise. The simulated currents were low-pass filtered and digitized at the same frequencies as those in the experiments and binned into an amplitude histogram. Then the program repeats a direct likelihood comparison between the simulated and experimental current amplitude distributions to find the best fitted values for the blocking kinetic parameters.

The ADA program showed that the off-rate of 1-AC block is 3-fold slower than that of 9-AC and the on-rate of 1-AC is ~3-fold faster than that of 9-AC. The voltage-dependences of on- and off- rates of 1-AC are similar to those of 9-AC, respectively. These suggest that 1-AC and 9-AC block CFTR channel by binding to a common binding site which should be modeled by a combination of a positive charge tightly surrounded by hydrophobic residues.

1670-Pos

Effects of Aromatic Carboxylic Acids on Genistein- and Curcumin- Potentiated G551D-CFTR

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The Cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel plays an important role in salt and water transport across epithelia and defective function due to mutations in the CFTR gene cause cystic fibrosis (CF). The glycine-to-aspartate missense mutation at position 551 (G551D) is the third most common CF-associated mutation and G551D-CFTR is characterized by a very low open probability despite of its normal trafficking to the plasma membrane.

Numerous small molecules have been shown to increase the activity of G551D-CFTR presumably by binding to the CFTR protein. Among the many G551D-CFTR potentiators, a bioflavonoid found in legumes, genistein is perhaps the most extensively studied. More recently it was reported that a component of the spice turmeric, curcumin strongly activated G551D-CFTR. However, The mechanism through which these compounds increase G551D-CFTR activity is still unclear. On the other hand, we have previously reported that anthracene-9-carboxylate (9-AC) showed an inhibitory effect and an potentiation effect on CFTR channels by binding to two chemically distinct sites for each effect (Ai *et al,* 2004).

In this study, we made a functional probing on genistein-potentiated G551D-CFTR and curcumin-potentiated G551D-CFTR using 9-AC and its positional isomer, anthracene-1-carboxylate (1-AC). In wild type- (WT-) CFTR, 9-AC induced a large voltage-independent enhancement and a voltage-dependent inhibition in the whole-cell (WC) current. 1-AC induced a smaller enhancement and a larger voltage-dependent block in WT-CFTR WC currents in compared with 9-AC. In the other hand, both 9-AC and 1-AC induced only a weak voltage-dependent inhibitions in genistein-potentiated G551D-CFTR WC currents whereas, in curcumin-potentiated G551D-CFTR WC currents, 9-AC and 1-AC induced similar effects to those in WT-CFTR. These suggest that curcumin and genistein potentiated G551D-CFTR via different mechanisms. We speculate that genistein-potentiated and curcumin-potentiated G551D-CFTRs might have different protein conformations.

1671-Pos

Optimization of the NBD1 Site Improves the Function of G551D-CFTR Channels

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CFTR chloride channels comprise two nucleotide binding domains (NBD1 and NBD2). Mutations (for example, G551D) that impair CFTR function result in the lethal genetic disease cystic fibrosis (CF). It's established that the opening and closing of CFTR are mainly controlled by ATP binding and hydrolysis respectively in NBD2 while ATP binding at NBD1 can modulate the stability of the open state. Using a non-hydrolytic ligand MgPPi, we locked open CFTR channels with mutations at NBD1. We found that two W401 mutations significantly increased the lock-open time (W401F: 72 ± 3s; W401Y: 51 ± 6s; WT: 27 ± 2s). These gain-of-function mutations are unexpected since the W401-equivalent residue Y1219 in NBD2 can be effectively replaced by tryptophan but not phenylalanine. As the ATP molecule bound in NBD1 may interact with NBD2's signature sequence (LSHGH), we extended our study to this region. We found that reverting the histidine residue 1348 to the canonical glycine (H1348G) similarly stabilized the lock-open state ($\tau = 67 \pm 8$s). Once W401F, H1348G, or W401F/H1348G mutations were incorporated into G551D channels which no longer respond to ATP, the mutant channels become ATP-responsive with basal activity increased by ~10-fold for W401F/G551D-, ~4-fold for H1348G/5G551D-, and ~25-fold for W401F/H1348G/G551D-channels. The increased