show that CLC-K channels are also inhibited at basic pH with almost complete block at pH 11. Based on the structure of a bacterial homologue, we mutated all external, basic, titratable residues of ClC-Ka (Arg, Lys, His, Cys, Tyr) and identified K165, a highly conserved residue in the extracellular vestibule of the channel, as the only candidate to be responsible for basic pH modulation. Among the mutants K165A, K165C, K165H, K165Q, and K165R, only K165R yielded (small) currents, with a weakened sensitivity to basic pH. We obtained functional recovery of K165C by reaction with the positively charged MTSEA reagent. Reversed voltage dependence of the alkaline pH effect on MTSEA-modified K165C channels compared to WT, supported the involvement of K165 in the pH modulation. The homologue K165C mutant of rat ClC-K1 yielded currents which were less sensitive to basic pH than WT CLC-K1 and MTSEA-modified CLC-K1-K165C channels recovered the WT sensitivity to basic pH, confirming that block of CLC-K channels at alkaline pH is mediated by the deprotonation of the pore lysine K165.

2791-Pos Board B561

Fluoride Transport in a Strange Subclass of Bacterial CLC Proteins

Randy Stockbridge, Christopher Miller.
HHMI, Brandeis University, Waltham, MA, USA.

Among prokaryotes, CLC-type Cl^- channels and transporters are phylogenetically abundant but functionally obscure. Enteric bacteria such as *E. coli* are known to use CLCs in extreme acid resistance, but many bacteria have several CLC genes from far-flung branches of the phylogenetic tree. We have been studying an evolutionarily distant CLC subclass that in sequence alignments lacks the canonical, mechanistically crucial central serine, which coordinates the central Cl^- ion in *E. coli* CLC-ec1 and other well-studied homologues. The protein-level function of these ''strange'' CLCs, whose genes have recently been shown to be specifically upregulated by F^- ion (J.L. Baker et al., in press), is unknown. Here we describe the overexpression, purification, and functional reconstitution of several CLC homologues that appear to protect diverse bacterial species from F^- toxicity. These ''fluoride-CLCs'' catalyze robust F^- transport in liposomes, as assayed by osmotic responses, F^-/Cl^- exchange, planar bilayer recording, and ^{19}F-NMR. We are currently carrying out a detailed characterization of one of these homologues, CLC-ps from the plant pathogen *Pseudomonas syringae*, including quaternary architecture, anion selectivity, transport rate and mechanism, and the presence or absence of H^+ coupling.

2792-Pos Board B562

Age-Dependence of Chloride Currents in Muscle Fibers from Control and Myotonic (HSA^{LR}) Mice

Carl H. Yu, Marino DiFranco, Marbella Quinonez, Julio L. Vergara.
UCLA, Los Angeles, CA, USA.

The transgenic mouse HSA^{LR} has been proposed as a model for myotonic dystrophy type 1, capable of recapitulating the human pathology, including the loss of chloride currents (I_{Cl}). Since the structural organization of the transverse tubular system (TTS) is not completed until ~3 weeks after birth, and the functional expression of ClC-1 channels may depend on this, we wanted to investigate whether the reduction of I_{Cl} in HSA^{LR} animals, compared to that in normal controls, changes with age. To this end, we performed a longitudinal study of the evolution of I_{Cl} and TTS voltage transients (using the potentiometric dye di-8-ANEPPS) in fibers from 2, 3, 4, 16 and 26 weeks old animals. I_{Cl} and optical signals were simultaneously acquired in fibers under voltage-clamp conditions using a 3-pulse protocol and a 2-microelectrode system. The fibers were bathed in 156 mM TEA-Cl and internally equilibrated with 70 mM Cl. The characteristics of I_{Cl}, and their effects on di-8-ANEPPS transients, were predicted using a radial cable model of the TTS. Our results show that, whereas fibers from 2-week old HSA^{LR} mice displayed peak I_{Cl} (for a -120 mV pulse) of -110 ± 50 $\mu A/cm^2$ (n=8), their control counterparts' were -510 ± 80 $\mu A/cm^2$ (n=6). Furthermore, the magnitude of I_{Cl} increases with age in both animal strains, and by 16 weeks they reach similar levels (>750 $\mu A/cm^2$). Thus, a reduction in I_{Cl} is significant in very young HSA^{LR} specimens, but not apparent in mature animals. We also verified that in HSA^{LR} animals the gain in total I_{Cl} with age is accompanied by the expression of functional ClC-1 channels in the TTS system. This work was supported by NIH grants AR047664, AR041802, and AR054816. HSA^{LR} mice were kindly provided by Dr. C. Thornton, University of Rochester.

2793-Pos Board B563

Functional Regulation of ClC-3 in the Migration of Vascular Smooth Muscle Cells

Sindura B. Ganapathi[1], Shun-Guang Wei[2], Fred S. Lamb[3], Stephen B. Shears[1].
[1]National Institutes of Environmental Health Sciences, Research Triangle Park, NC, USA, [2]Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA, USA, [3]Vanderbilt University, Nashville, TN, USA.

Migration of vascular smooth muscle cells (VSMC) into neointima contributes to hypertension, atherosclerosis, and restenosis. Cell migration requires coordinated plasmalemmal fluxes of water and ions. Here, we show that regulation of transmembrane Cl^- flux significantly impacts the migration of VSMC. Gene disruption of ClC-3, a Cl^- channel/transporter, halved the rate of cell migration in transwell assays. Functional regulation of plasmalemmal Cl^- current by ClC-3 was studied by electrophysiological recordings. Raising intracellular $[Ca^{2+}]$ from zero to 0.5 μM in wild-type cells stimulated a Cl^- current ($I_{Cl.Ca}$) that was reduced approximately 40% upon CaMKII inhibition by 100 μM KN-93, or by application of 10 μM inositol-3,4,5,6-tetrakisphosphate, a cellular signal that specifically prevents CaMKII from activating $I_{Cl.Ca}$. Thus, $I_{Cl.Ca}$ comprises two components, one directly activated by Ca^{2+}, and another that requires CaMKII. $I_{Cl.Ca}$ was 50% smaller in ClC-3 null cells compared to wild-type VSMC; neither KN-93 nor inositol-3,4,5,6-tetrakisphosphate affected $I_{Cl.Ca}$ in ClC-3 null VSMC. Thus, the CaMKII-mediated component of $I_{Cl.Ca}$ in VSMC is dependent upon ClC-3. Both Ca^{2+}-dependent and CaMKII-dependent forms of $I_{Cl.Ca}$ were strongly inhibited by niflumic acid, a Cl^- channel blocker, but, significantly, that drug only inhibited migration of wild-type and not ClC-3 null VSMC. Moreover, a cell-permeant, bio-activatable analogue of inositol-3,4,5,6-tetrakisphosphate inhibited migration in wild-type, but not ClC-3 null cells. Our work describes for the first time a specific role of ClC-3 in VSMC migration, thereby revealing new therapeutic directions in vascular remodeling diseases.

2794-Pos Board B564

Disease-Related Modification of Chloride Conductance in Skeletal Muscle of Dystrophic Mice: Expression of CLC-1 Channel and Role of Inflammation and Oxidative Stress-Related Signaling

Giulia M. Camerino, Anna Cozzoli, Roberta F. Capogrosso, Maria M. Dinardo, **Annamaria De Luca**.
Unit of Pharmacology, Dept. Pharmacobiology, University of Bari, Italy.

A decrease in resting chloride channel conductance (gCl) occurs in dystrophin-deficient myofibers of mdx mouse as a consequence of both spontaneous degeneration, as in diaphragm (DIA), or exercise-induced damage as in fast-twitch EDL muscle (De Luca et al., J. Pharmacol. Exp. Ther. 2003). Alterations in gCl, in parallel with markers of inflammation, can be contrasted by small restoration in dystrophin expression (De Luca et al., Neurobiol Dis, 2008). We focused on the molecular mechanisms underlying gCl impairment and its relation to primary defect. Preliminary qRT-PCR experiments showed a 30-35% reduction of CLC-1 mRNA in both DIA and EDL muscles of mdx mice, irrespective to exercise regimen. The reduction was consistent with the impairment of gCl detected in concomitant electrophysiological experiments in DIA and EDL myofibers; however the selective alteration of gCl in exercised mdx EDL muscle remained unexplained. Further experiments are ongoing to evaluate the possible outcome of pathology and mechanical stress on CLC-1 channel protein level and on the expression of other chloride channel types. Recent results showed that in vivo inhibition of angiotensin (Ang)-II contrasts the decrease in gCl in mdx EDL muscle, disclosing a possible role of this pro-inflammatory and pro-oxidative mediator in chloride channel function (Cozzoli et al., Pharmacol Res 2011). The application of Ang-II to wt EDL fibers reduced gCl in a concentration-dependent manner, with a half-maximal concentration of 67nM. The effect was inhibited by the AT1-receptor antagonist losartan, as well as by the PKC-inhibitor chelerythrine, the antioxidant N-acetyl-cysteine and the inhibitor of NADPH-oxidase apocynin. The results demonstrate that CLC-1 channel expression is affected in dystrophinopathies and that further modulation of gCl is related to inflammation and oxidative stress in skeletal muscle.

2795-Pos Board B565

Single Molecule Optical Determination of Bestrophin Stoichiometry

Shashank Bharill, Ehud Y. Isacoff.
University of California Berkeley, Berkeley, CA, USA.

Best macular dystrophy (BMD) is an autosomal dominant form of macular degeneration, linked to mutations in the BEST1 gene that encodes the calcium activated chloride channel, bestrophin-1. BEST1 belongs to the bestrophin family of anion channels. It is a 585 amino acid transmembrane protein localized to the basolateral membrane of the retinal pigment epithelium (RPE). Structurally, BEST1 has been suggested to have 4 transmembrane segments, with intracellular N and C termini. The stoichiometry of BEST1 is not clear, having been suggested to be dimers in hydrodynamic studies of porcine BEST1 in TritonX-100 and tetramers or pentamers in coimmunoprecipitation studies of human BEST1 (hBEST1). In this study, we employed our single molecule method to determine subunit number by counting the number of fluorescence bleaching steps of eGFP tagged hBEST1. The advantage of this method is that the counting can be done on the cell surface of live cells (Xenopus oocytes