746-Pos Board B501
Roles of Cytoplasmic Ions in Lysosomal Acidification
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The lysosome is the terminal organelle in the endocytic pathway; its role is to degrade and recycle macromolecules. To function properly it must maintain an acidic pH. A V-type ATPase is necessary to drive protons into lysosomes, but the action of this pump generates a large voltage across the membrane, which limits further pumping. Fluxes of ions are therefore necessary to dissipate the electrochemical gradient, referred as the ‘‘counterion pathway.’’ It remains poorly understood which ions contribute to this pathway and facilitate net acidification of the lysosome. Our goal is to investigate the mechanism underlying the acidification of lysosomes and to evaluate the role of ClC-7, a lysosomal H^+/Cl- exchanger in the acidification process. We have probed the effects of external ion concentrations on acidification in isolated lysosomes using fluorescent ion- or voltage-sensing probes to measure internal pH or changes in membrane voltage.
Using isolated lysosomes we demonstrate that the presence of external anions facilitates lysosomal acidification, suggesting a role in the counterion pathway. The anion specificity is similar to the anion selectivity observed for members of the ClC family (Cl->Br->I->NO3-). In contrast, the presence of cytoplasmic like concentrations of external K^+ doesn’t facilitate acidification; rather, at these levels (100mM), K^+ ions induce alkalinization, which is enhanced in the presence of Valinomycin. Using the DisC3(5) membrane potential dye we monitored lysosomal membrane potential while varying external [K^+]; we estimate the relative K^+ permeability with and without Valinomycin. Finally, we used the null point titration approach to estimate the luminal concentration of ions, obtaining values in a range similar to those found using pH changes. This work helps constrain models of lysosomal acidification and suggests a limited role for K^+ in the process.

747-Pos Board B502
Involvement of Barttin Subunit in Pharmacological Potentiation of CLC-K Channels Expressed in Xenopus Oocytes
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CLC-K Cl^- channels of the CLC family are almost exclusively expressed in kidney and inner ear epithelia where they participate in NaCl reabsorption and endolymph production, respectively. Both in the kidney and in the inner ear CLC-Ks co-assemble with a small beta subunit, barttin, that affects trafficking, stability, and gating of CLC-Ks by unknown interaction mechanisms. Mutations in ClC-Kb and barttin cause Bartter syndrome. Previously, the search for potential therapies led to identify niflumic acid (NFA), a drug belonging to the fenamate class, as the most potent human CLC-K activator so far known (Liantonio et al, 2006. *Mol Pharmacol* 69:165; Picollo et al, 2007. *J Membr Biol* 216:73). CLC-K isoforms expressed in *Xenopus* oocytes show peculiar NFA responses: hCLC-Kb is activated by $[NFA]_{ext}$ up to 2 mM, hCLC-Ka is potentiated by $[NFA]_{ext} \leq 0.5$ mM whereas it is inhibited by higher $[NFA]_{ext}$, and rat CLC-K1 is blocked at all $[NFA]_{ext}$ tested. We recently identified the extracellularly accessible residue F256 that when mutated to Ala hugely increases the activating effect of NFA on CLC-Ka. Furthermore, the F256A mutation confers onto CLC-K1 a transient potentiation induced by NFA. Interestingly, activation was found only when CLC-K1/F256A was co-expressed with barttin. Based on a model of barttin’s structure (Estevez et al, 2001. *Nature* 414:558), a mutagenic screen of the extracellular loop led us to identify four residues (R30, Q32, F37, and Y38) that when mutated abolished CLC-Ka potentiation by NFA, suggesting the involvement of the subunit in this process. Thus, in addition to barttin’s known roles, it participates in the pharmacological modulation of CLC-K channels.

748-Pos Board B503
Reduced Current Density and Surface Expression of a CLCN1 Mutation Causing Dominant or Recessive Myotonia in Costa Rica
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Mutations in CLCN1 coding for the muscle ClC-1 Cl- channel lead to dominant (Thomsen’s disease) of recessive (Becker’s disease) myotonia. Here we characterized several CLCN1 mutations identified in Costa Rica myotonia patients: Q412P (Morales 2008. RevBiolTrop 56:1), R105C, Q154R, and F167L (R105C and F167L have been described earlier in German families (Meyer-Kleine 1995. AmJHumGenet57:1325, Zhang 2000. Neurology54:937). In different families, the Q412P mutation was found to be inherited both in recessive and dominant fashion. We studied the Cl- currents generated by these mutants in Xenopus oocytes using electrophysiological techniques, and for the Q412P mutant also surface membrane expression. Assaying for fast and common gating properties and single channel conductance, we found that none of the mutations exhibited significant alterations of gating parameters or conductance. In fact, mutations R105C, Q154R, and F167L were indistinguishable from WT ClC-1 (in agreement with earlier studies for R105C and F167L (Desaphy 2013, ExpNeurol 248:530). However, mutation Q412P displayed a dramatically reduced current density and a reduced surface expression. However, mimicking the heterozygous status of patients by co-injecting WT and mutant RNA in a 1:1 ratio did not reveal a significant reduction of current density compared to the injection of the half amount of WT. Our results indicate that Q412P does not exert a dominant negative effect on WT and that the pathophysiology of the R105C, Q154R, and F167L mutations is not due to reduced open probability or single channel current, but that these mutations must affect mechanisms that are not well reproduced in a non-skeletal muscle expression systems. The lack of dominance of Q412P might be due to early protein degradation (before being able to interact with WT subunits) or to a silent phenotype in WT/Q412P heteromers.

749-Pos Board B504
Role of Candidate Counterions in Clathrin Coated Vesicle Acidification
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Organelles such as endosomes and lysosomes maintain a characteristic internal pH essential for their function. This acidic luminal pH is generated and maintained by the V-type ATPase, a protein that hydrolyzes ATP to pump protons (H^+) into the organelle against their electrochemical gradient. H^+-pumping generates a voltage across the membrane and an additional ion, known as a counterion, must move to dissipate the charge buildup and allow additional proton pumping. The counterion could be an anion moving into the organelle, a cation exiting, or a combination of both. Though progress has been made in probing the counterion pathways involved in lysosomal acidification, counterion movement in endosomes is poorly understood, partly because of the difficulty of isolating a homogeneous endosome population. However, clathrin-coated vesicles (CCVs), a subpopulation of early endosomes, can be more readily obtained. We have isolated highly-enriched bovine brain CCVs to probe their acidification mechanism and the role of counterion movement. We find that CCVs acidify robustly upon the addition of ATP when monitored with the pH-responsive fluorophore acridine orange. This acidification is completely dependent on external Cl^-, although some other monovalent anions can partially substitute. Further, acidification is essentially independent of the external monovalent cation, suggesting a prominent role for Cl^- transport in the counterion pathway in these organelles. We continue to investigate the identity and mechanistic transport properties of the Cl^- dependence in CCVs, ultimately hoping to understand the role of Cl^- transport in endosomal acidification using CCVs as a model system.

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Characterizing ATP Permeation through the Voltage-Dependent Anion Channel VDAC
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Voltage-dependent anion channels (VDAC) mediate the transfer of metabolites such as ATP, ADP, and NADH across the outer mitochondrial membrane of all eukaryotic cells. The open state is selective for anions, while the partially closed state is selective for cations. The x-ray crystal structure of VDAC from mouse (mVDAC1) (Ujwal et al. PNAS 2008) made it possible to study the molecular workings of this channel in unprecedented detail.
Previously, we used continuum electrostatic calculations to show that mVDAC1 is anion selective suggesting that the x-ray crystal structure represents the open state of the channel (Choudhary et al. JMB 2010). However, the hallmark of the open state is high ATP flux. The open channel passes millions of ATP molecules per second, while the closed state exhibits no detectable