advantage of this we used electrophysiological techniques on HEK293 cells expressing Piezo channels to investigate the effects of Phospholipase C (PLC) activating pathways and specifically phosphoinositide depletion. PLC activation, which converts PIP_2 into IP_3 and DAG, inhibited mechanically activated currents in Piezo expressing cells. Phosphoinositide depletion using rapidly inducible lipid phosphatases also inhibited Piezo channel activity, but without generating IP_3 or DAG. Our data provides evidence that phosphoinositides are involved in the regulation of Piezo channels, thus our study contributes to the understanding of lipid regulation of mechanically gated channels.

3797-Pos Board B525
Linolenic and Linoleic Acid Induce the Opening of Connexin 43, 46 and 50 Hemichannel in Human Hela Cells
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Abstract: Connexin (Cx) hemichannels (HC) located at the plasma membrane have a very low open probability, but under physiological conditions they can briefly open, allowing passage of both ions and small signaling molecules, thereby participating in the control of diverse cellular responses. Cx43, 46 and 50 are expressed in human lens and are essential to maintaining its transparency, since mutations in the genes coding for these Cxs have been identified, inducing the development of different types of cataracts. However, non-congenital cataracts may result from consumption of polyunsaturated fatty acids (PUFAs). While the consumption of omega-6 PUFAs, such as linoleic acid (ω-6LA) has been associated with development of cataracts, intake of omega-3linolenic acid (ω-3LA) has been associated with their decline. Here, we report the effects of ω-6LA and ω-3LA on HCs in HeLa cells that express Cx43, Cx46 or Cx50. Bath application of ω-6LA or ω-3LA induces opening of HCs (measured as Ethidium uptake) formed by Cx43, Cx46 or Cx50in HeLa cells and also in human lens epithelial cell line (HLE) that expresses endogenously Cx46 and Cx50 HCs, in a concentration- dependent manner and inhibited by HC blockers such as Lanthanum. Both PUFAs also increased the membrane current mediated by these HCs measured by patch clamp assays in the whole cell configuration in both cell types. Since PUFAs have demonstrated cytotoxic effects in HLE and also in organotypic cultures of bovine lens epithelial cells, their effect on HCs might be relevant to development and prevention of cataracts.

3798-Pos Board B526
Enhancing the ATPase Activity of the Cystic Fibrosis Transmembrane Conductance Regulator CFTR in Live Cells Reduces Chloride Flux
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Maintaining the fluid balance in tissues such as the lung airways, the intestine, and many exocrine glands is of vital importance. This task is fulfilled essentially by the cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7), a channel for passive diffusion of chloride and bicarbonate. Deregulation of CFTR causes multiple disorders, the most prominent of which is cystic fibrosis (CF). CFTR is also implicated in autosomal dominant polycystic kidney disease and secretory diarrheas. Owing to the increasing number of diseases recognized to be associated with CFTR dysfunction, the interest in CFTR potentiators and inhibitors has grown. However, their function at the molecular level is not yet understood. On the one hand, this is due to the complex function of CFTR which, as a member of the ATP binding cassette (ABC) transporter family, is the only known ion channel that links ATP binding and hydrolysis to channel gating; on the other hand, it is due to fact that chloride efflux is generally measured in cells, whereas ATP hydrolysis was so far measured in reconstituted proteoliposomes exhibiting significant functional attrition. Here we take a new approach to answer the intriguing question as to whether chloride channel inhibitors and potentiators enhance or reduce ATP hydrolysis by CFTR. For this purpose we measured ATPase activity and chloride efflux, both, in live cells, under similar experimental conditions. ATPase activity was measured by microphsiometry using silicon chip technology and anion flux was measured using an iodide electrode. Most importantly we demonstrate that chloride flux inhibitors enhance ATPase activity of CFTR, whereas chloride channel potentiators do not stimulate ATPase activity, but tend to reduce cell metabolism. These findings have implications for the synthesis of new potentiators and inhibitors.

3799-Pos Board B527
Mechanism of CLCA1-Mediated CACC Activation in Chronic Lung Diseases
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CLCA1 plays a central role in chronic inflammatory lung diseases (asthma and COPD). Overexpression of CLCA1 drives mucous overproduction and activates a Ca-activated chloride channel (CaCC), both through unknown mechanisms. Here we show that CLCA1 is a soluble secreted protein that gets cleaved into two fragments upon secretion. The cleavage is carried out by a novel internal zincin metalloprotease, located in the N-terminal domain. This self-cleavage reaction is required for the activation of chloride currents, as mutants with an impaired active site or cleavage site failed to increase currents above background levels. We observed that the N-terminal cleavage fragment is necessary and sufficient to activate the unknown CaCC, without directly proteolytically cleaving the channel. These results suggest a direct interaction between the channel and its modulator, CLCA1. Our current focus is on showing physical and functional association between these two molecules and identifying the CaCC by using pulldown experiments and functional patch clamp assays in the presence of siRNA and channel blockers. Latest results will be presented.

3800-Pos Board B528
The Maguk Scaffolding Protein CASK Regulates TMEM16A Channel Function by Phosphorylation
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Ca-activated Cl channels (CaCCs) encoded by TMEM16A (Ano1) play essential roles in many physiological processes including epithelial fluid secretion, gut motility, and smooth muscle tone. Using a quantitative SILAC proteomics approach, we discovered that CASK, a unique MAGUK protein containing an N-terminal CaM-kinase homology domain, is very highly enriched in the TMEM16A interactome. CASK contains multiple protein-protein interaction domains linking receptors and signaling molecules at membrane-cytoskeletal interfaces and has unique protein kinase activity that is inhibited by Mg^{2+}. Here we show that CASK co-immunoprecipitates with TMEM16A and regulates the voltage-dependence of TMEM16A in Mg^{2+}-sensitive manner. In the presence of 1 μM free intracellular Ca^{2+}, decreasing intracellular Mg^{2+} from 2 mM to 0 mM shifts the conductance-voltage (G-V) relationship 98.4 mV in a hyperpolarizing direction without changing GMAX or Ca^{2+} sensitivity. The Mg^{2+}-dependent shift in the G-V curve is dependent on CASK protein kinase activity because shRNA-knockdown of CASK or replacement of ATP with AMP-PNP eliminates the shift. Mutation of S639 in TMEM16A identifies this amino acid is the site phosphorylated by CASK. The G-V curve of the S639A mutant is unaffected by Mg^{2+} or ATP and has a V0.5 value similar to WT in the presence of 2 mM Mg^{2+} [WT(+Mg^{2+}) 98.6 mV; S639A(0Mg^{2+}) 78.8 mV]. The G-V curve of the phosphomimetic S639E mutant is unaffected by Mg^{2+} or ATP, but the V0.5 value is similar to WT in the absence of Mg^{2+} [WT(0 Mg^{2+}) −3.5 mV; S639A(2 Mg^{2+}) 7.7 mV]. Inhibitory autocamtide-2 (AIP-2), a CaMKII inhibitory substrate that also interferes with the catalytic activity of CASK in the internal solution efficiently abolishes the effect of Mg^{2+} on TMEM16A currents. These data suggest that CASK may regulate TMEM16A channels, although the physiological significance in cells remains to be determined.

3801-Pos Board B529
Modulation of the Voltage-Gated Proton Channel Hv1 by Small Gating-Modifier Ligands
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The human voltage-gated proton channel Hv1 is activated by depolarization and intracellular acidification to maintain the cell's neutral pH. It belongs to voltage-gated cation channels, consisting of a typical voltage sensor domain (VSD) but lacking a canonical pore domain. These channels assemble as dimers and recent data indicate strong cooperativity between the two subunits. Recently, guanidine derivatives were found to act as open channel blockers on the Hv1 VSD. Here we show that the gating modifiers NH29 and NH34, two diphenylamine carboxylate derivatives, act as Hv1 channel openers. NH29 was recently shown to exert opposite gating-modifier effects on the VSD of Kv7.2 potassium channels and TRPV1 cationic channels operating as opener and blocker of Kv7.2 and TRPV1 currents, respectively. Both compounds increase the Hv1 current amplitude by more than 2.5-fold at + 50 mV and produce a left-shift in the voltage dependence of channel activation by about −15 mV. We found that NH29 and NH34 change the proton selectivity of Hv1 channels and produce a left-shift of about −10 mV in the reversal potentials both in symmetrical (pH 7.0) and asymmetrical pH solutions (pHi 5.5/pHo 7.0). Our results also show that the mutation R211S in S4 profoundly affects the modulatory properties of the compounds. The S4 residue R211 was previously suggested to be crucial for Hv1 proton selectivity by interacting with D112 in S1. In mutant R211S, NH29 does not act anymore as an opener but rather inhibits Hv1 currents. Other Hv1 mutations are currently tested in order to determine how Hv1 channels interact with these small ligands and to elucidate Hv1 selectivity and gating mechanisms.