importance of the resulting rise in $[\text{Ca}^{2+}]_i$ is universally acknowledged, the biophysics of the $\text{Ca}^{2+}$ current responsible for the effect are poorly understood, largely because traditional methods of measuring $\text{Ca}^{2+}$ permeability cannot be applied to P2X7 receptors. Here we use an alternative approach, called patch-clamp photometry, to quantify the agonist-gated Ca$^{2+}$ current of recombinant P2X7 receptors of dog, guinea-pig, human, monkey, mouse, rat, and zebrafish. We find that the magnitude of the Ca$^{2+}$ current depends on the species of origin, the splice variant, and the concentration of the purinergic agonist. Surprisingly, murine P2X7Rs transduce smaller Ca$^{2+}$ currents than do their P2X7aR counterparts, despite having identical pore-forming domains. We also measured a significant contribution of Ca$^{2+}$ to the agonist-gated current of the native P2X7Rs of mouse and human macrophages. Our results provide cross-species quantitative measures of the Ca$^{2+}$ currents of P2X7 receptors for the first time, and suggest that the cytoplasmic domains play a meaningful role in regulating the flow of Ca$^{2+}$ through the channel.

1449-Pos  Board B400
Ion Accumulation and Depletion in Patch Clamp Experiments
Gilman E.S., Toombes, Mufeng Li, Shai D. Silberberg, Kenton J. Swartz. Molecular Physiology and Biophysics Section, NINDS, Bethesda, MD, USA. The flow of ions through channels and transporters depends sensitively on the concentration of the ionic species and voltage at the membrane. In the widely used patch-clamp technique, voltage and current are measured using electrodes in the pipette and bath that are distant from the membrane. Although the importance of voltage differences between the membrane and electrodes is widely appreciated, concentration gradients arising from the flow of ions are often neglected. In this study, we modeled the voltage and ion concentrations during patch-clamp experiments using the Nernst-Planck equation and we derived simple formulae for estimating the timescale and extent of ion accumulation and depletion. For excised patch experiments, ions crossing the membrane can directly diffuse into or out of the patch pipette and ion concentrations stabilize on the millisecond timescale after a change in membrane current. In contrast, in whole-cell experiments the cytosol acts as a reservoir and ion concentrations change on the timescale of seconds. In either configuration, ion accumulation or depletion at steady-state depends primarily on the electrode access resistances and currents carried by each ionic species. As a practical illustration, simulations were performed for bi-ionic protocols previously used to characterize the dynamic ionic selectivity of P2X and TRPV channels. Importantly, even when the net current was small and the membrane voltage effectively clamped, ion accumulation and depletion could cause significant, time-dependent changes in current resembling reported examples of “pore dilution”. Thus, limitations for clamping ion concentrations should be considered when performing and interpreting patch-clamp experiments.

1450-Pos  Board B401
Hexadecanol Reverses Ethanol Induced Tadpole Anesthesia and Raises Critical Temperatures in Isolated Plasma Membrane Vesicles
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was studied using whole cell currents. Channels containing a single Asp to Arg mutation were expressed in Xenopus oocytes and currents were recorded by using two-microelectrode voltage clamp. [NaCl], was elevated by using microelectrodes filled with 2M NaCl. All mutant channels other than D757R responded to intracellular NaCl loading similar to WT channels. D757R channels were activated by 1 mM NFA, confirming their functional expression. Similar results were obtained for whole cell currents recorded in HEK cells expressing WT or D757R Slo2.1 channels. With 70 mM NaCl in the pipette solution, WT Slo2.1 current increased 30-fold, whereas D757R currents were not activated. Both WT and D757R channel currents were increased by ≤50-fold by 0.5-1 mM NFA. Together these findings indicate that a crucial soluble cofactor required for activation of channels is lost upon patch excision and that Asp757 is the primary determinant of Na⁺ activation of Slo2.1.

1454-Pos Board B405
Electrophysiological Characterization of TMEM16A
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Sweating is an essential physiological process to regulate body temperature in humans and various disorders are associated with dysregulated sweat formation. Primary sweat secretion in human eccrine sweat glands is initiated by Ca²⁺-activated Cl⁻-channels (CaCC). Recently, members of the TMEM16 family were identified as CaCCs in various secretory epithelia, however, their molecular identity in sweat glands had to be elucidated. Here we investigated the function of TMEM16A in sweat glands. Gene expression analysis revealed that TMEM16A is expressed in human NCL-SG3 sweat gland cells as well as in isolated human eccrine sweat gland biopsies samples. Sweat gland cells express several previously described TMEM16A splice variants, as well as one novel splice variant, TME-M16a(ace3) lacking the TMEM16A-dimerization domain. Chloride-flux assays using halide-sensitive YFP revealed that TMEM16A is functionally involved in Ca²⁺-dependent Cl⁻-transport in NCL-SG3 cells. Reombinant expression in NCL-SG3 cells showed that TMEM16a(ace3) is forming a functional CaCC, with basal and Ca²⁺-activated Cl⁻-permeability distinct from canonical TME-M16a(ace). Our results suggest that various TMEM16A isoforms contribute to sweat gland-specific Cl- transport providing opportunities to develop sweat gland-specific therapeutics for the treatment of sweating disorders.

HEK cells expressing TMEM16A were investigated by high throughput giga seal patch clamping with the SyncroPatch 384P. Currents were activated by internal Ca and could be blocked by Niflumic Acid.

1455-Pos Board B406
Photodynamic Modification of Sea Urchin sPHC Channel
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The hyperpolarization activated cyclic nucleotide gated ion channels (HCN channels) are nonspecific cation channels that play vital role in the generation of rhythmic activities in the brain and the heart. Among the various members of HCN channels, sPHC channel is unique in that it undergoes rapid inactivation following a hyperpolarizing voltage step and cAMP binding abolishes the inactivation. Previously, we had shown that mouse HCN2 channel can be modified by a photodynamic process that involves photosensitizer, oxygen and laser pulses. Here, we asked whether similar photodynamic process could modify sPHC channel. We applied 1mM FITC-conjugated cAMP to the intracellular side of the membrane patch. Application of short laser pulses abolished sPHC inactivation. The channel opening after the laser pulses can be fitted with a side of the membrane patch. Application of short laser pulses abolished sPHC inactivation. 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