when coexpressed with N228D as well as FaPIP2;1, ii) the coexpression of FaPIP2;1-FaPIP1;1 resulted in higher Pf than N228D-FaPIP1;1, iii) pH sensitivity measured in terms of EC50 are the same for both coexpressions. Our results allows us to hypothesized that the Pf showed by the coexpression of FaPIP1;1-N228D could represent FaPIP1;1 water transport capacity, being N228D inactive as a water channel. The contribution of FaPIP1;1 is relevant in terms of increasing faster water adjustments in the PM and allowing blocking the pores at a more physiological pH.

**3242-Pos Board B397**

**CALHM1 is an Extracellular $Ca^{2+}$- and Voltage-Gated ATP Permeable Ion Channel**

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We identified CALHM1 as a pore-forming subunit of a plasma membrane ion channel with weak ion selectivity and unique coupled allosteric gating regulation by voltage and extracellular $Ca^{2+}$ ($Ca^{2+}_o$) (*PNAS* 109: E1963 (2012)). CALHM1 is expressed in mouse cortical neurons where it accounts for low $[Ca^{2+}]_o$-enhanced conductance and action potential firing. We recently determined that a CALHM1 channel is a hexamer with an estimated effective pore diameter ~14Å.Extracellular adenosine 5'-triphosphate (ATP) plays critical roles in physiological and signal transduction processes. We examined whether ATP can permeate CALHM1 channels. Reducing $[Ca^{2+}]_o$ to activate CALHM1 induced ATP release from hCALHM1-expressing HeLa and COS-1 cells, and *Xenopus* oocytes. Neither CALHM1 expression nor lowering $[Ca^{2+}]_o$ caused cell damage. Involvement of other possible mechanisms was ruled out because ATP release was unaffected by Brefeldin A (vesicular release), DCPIB (volume-sensitive $Cl^-$ channels), A438079 (P2X7 receptors), heptanol and carbenoxolone (connexins and pannexins). In contrast, ruthenium red (RuR), which inhibits CALHM1 currents, abolished low $[Ca^{2+}]_o$-evoked ATP release. Thus, CALHM1 expression induces a novel ATP permeability. $Ca^{2+}_o$ inhibited ATP release with $IC_{50}$ = 495 μM and Hill coefficient of 1.9, kinetic properties similar to those of its gating regulation. Membrane depolarization activates CALHM1 channels in normal $[Ca^{2+}]_o$. hCALHM1-expressing but not mock-transfected cells released ATP in response to high $[K^+]_o$-induced depolarization in normal $[Ca^{2+}]_o$, which was inhibited by RuR but not by connexin and pannexin-1 blockers. Thus, regulation of ATP release is correlated with the gating properties of CALHM1 channels, indicating that the CALHM1 channel is the conduit for ATP release. These results demonstrate that CALHM1 is a voltage-gated ATP release channel that may contribute to ATP release in physiological and pathological conditions.

**3243-Pos Board B398**

**CALHM1 Ion Channel Mediates Purinergic Neurotransmission from Taste Buds to Gustatory Nerve Terminals during Sweet and Bitter Perception**

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Taste buds (TB), composed of three distinct types of cells (type I, II and III), sense taste compounds and transmit signals to afferent gustatory neural pathways. Neurotransmission of sweet and bitter tastes requires non-vesicular release from type II cells of adenosine 5'-triphosphate (ATP) which acts as a neurotransmitter to activate afferent neural pathways. However, how ATP is released is uncertain. We recently identified CALHM1 as an ATP-permeable ion channel, and CALHM1 was found to be expressed in primate TB. Therefore, we examined the possibility that CALHM1 mediates ATP release from type II cells during sweet and bitter perception. By *in situ* hybridization, *Calhm1* was expressed in mouse TB but not in surrounding epithelium. Loss of *Calhm1* signal in TB of *Skn-1a*$^{-/-}$ mice in which type II cells are developmentally absent demonstrates that *Calhm1* expression is confined to sweet/bitter-sensing type II cells. To examine CALHM1 function, we generated a constitutive *Calhm1*$^{-/-}$ mouse and verified loss of *Calhm1* expression in TB. *Calhm1*$^{-/-}$ mice were viable and fertile, with no overt morphological or marker-gene expression abnormalities in their TB. Knockout of *Calhm1* significantly reduced voltage-dependent currents in type II cells, which were inhibited by ruthenium red, a CALHM1 channel blocker, but was without effects on the excitability of taste cells to taste stimuli. Strikingly, taste-evoked release ATP release from TB was abolished in *Calhm1*$^{-/-}$ mice. Finally, *Calhm1* deficiency eliminated behavioral responses to sweet and bitter taste stimuli but did not impact salty and sour tastes. Thus, CALHM1 is an essential component of sweet and bitter perception as the neurotransmitter (ATP) release pathway.

**3244-Pos Board B399**

**Ion Conduction in the Stem of the $PA_{63}$ Channel of Anthrax Toxin during Lethal Factor (LF) Translocation**

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Protective antigen (PA), a critical 83kDa protein component of the anthrax toxin, forms channels in cell membranes which allow for the transport of two toxin components, lethal factor (LF) and edema factor (EF), into cells. The heptameric channel formed by $PA_{63}$ (the 63 kDa, C-terminal part of PA) is thought to be a mushroom-shaped structure, with seven phenylalanine residues forming a ring (known as the phenylalanine clamp) at the junction between the cap and the stem. It is known that when LF is driven through the channel by an applied ΔV across the membrane, the seal created by the phenylalanine clamp causes an essentially complete block of conduction, but it is not known whether the stem of the channel allows for any conduction when occupied by LF. We are studying the $PA_{63}$ channel at both the microscopic and the macroscopic level in an effort to determine how much conductance, if any, is allowed when the channel is occupied by LF and the phenylalanine clamp has been mutated to less bulky alanine residues. Preliminary data suggest that when LF is added to the *cis* compartment containing the mutant channel, the block in conductance (at positive voltages) may not be as complete as when the phenylalanine clamp is present, but it is nonetheless significant. If this is the case, then the electric field created by the ΔV across the membrane is focused at the phenylalanine clamp.

**3245-Pos Board B400**

**Magnesium-Dependent Modulation of Junctional Conductance and Gating Properties of Connexin36 Gap Junction Channels**

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Gap junction (GJ) channels formed by connexin36 (Cx36) play an important role in neuronal synchronization, and calcium oscillations in insulin-secreting beta cells. Here we describe a new form of plasticity of Cx36 GJ channels dependent on intracellular free magnesium ($[Mg^{2+}]_i$). We examined junctional conductance ($g_j$) and its dependence on transjunctional voltage ($V_j$) in HeLa and neuroblastoma N2A cells expressing Cx36 at different $[Mg^{2+}]_i$. A remarkable ~3.5-fold increase in $g_j$ was observed when $[Mg^{2+}]_i$ was reduced to 0.01 mM, and a reduction to ~1/5$^{th}$ of initial values when $[Mg^{2+}]_i$ was augmented to 5 mM; for $[Mg^{2+}]_i$ action $EC_{50}$= ~0.45 mM. By using a stochastic 16-state model of voltage gating, we demonstrate that lowered $[Mg^{2+}]_i$ increases open channel probability while enhanced $[Mg^{2+}]_i$ reduces it. Similar changes in conductance and $V_j$-gating are observed with MgATP or $K_2ATP$, which increases or decreases $[Mg^{2+}]_i$, respectively. Changes in phosphorylation of Cx36 or $[Ca^{2+}]_i$ are not involved in the observed Mg-dependent modulation of $g_j$. Magnesium ions permeate the channel and transjunctional asymmetry in $[Mg^{2+}]_i$ results in asymmetric $V_j$-gating. We propose that the lumen of Cx36 GJ channels contains binding site(s) for $Mg^{2+}$, and that $Mg^{2+}$ stabilizes a closed channel conformation. Conductance of GJs formed by Cx26, 32, 43, 45 and 47 expressed in HeLa cells are also reduced by increasing $[Mg^{2+}]_i$ above resting levels. However, none of these Cxs show increase in $g_j$ upon reduction in $[Mg^{2+}]_i$; thus, Cx36 is the only tested Cx sensitive to lowering of physiological levels of free $Mg^{2+}$. This novel $Mg^{2+}$-dependent modulation of Cx36 GJ channels can be important for changes in neuronal synchronization and insulin secretion under physiological and pathological conditions when ATP levels, and consequently $[Mg^{2+}]_i$, are modified.

**3246-Pos Board B401**

**Disruption of Salt Bridge Interactions Modifies Gating Kinetics of Connexin Hemichannels**

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Opening and closing of connexin hemichannels is involved in physiological paracrine signaling via release of ATP and other cytosolic molecules, but exacerbated hemichannel opening caused by mutations or pathological conditions is detrimental. The molecular mechanisms that control opening and closing of connexin hemichannels are poorly understood. The crystal structure of human