

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.  $[NaCl]_i$  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 >150-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  $Ca^{2+}$ -activated Cl<sup>-</sup> channels (CaCC). Recently, members of the TMEM16 family were identified as CaCCs in various secretory epithelia, however, their molecular identity in sweat glands remained elusive. 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 biopsy samples. Sweat gland cells express several previously described TMEM16A splice variants, as well as one novel splice variant, TMEM16A(ace3) lacking the TMEM16A-dimerization domain. Chloride-flux assays using halide-sensitive YFP revealed that TMEM16A is functionally involved in  $Ca^{2+}$ -dependent Cl<sup>-</sup> transport in NCL-SG3 cells. Recombinant expression in NCL-SG3 cells showed that TMEM16A(ace3) is forming a functional CaCC, with basal and  $Ca^{2+}$ -activated Cl<sup>-</sup> permeability distinct from canonical TMEM16A(ac). Our results suggest that various TMEM16A isoforms contribute to sweat gland-specific Cl<sup>-</sup> 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 384PE. Currents were activated by internal Ca and could be blocked by Niflumic Acid.

#### 1455-Pos Board B406

##### Photodynamic Modification of Sea Urchin spHCN 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, spHCN 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 spHCN channel. We applied 1 $\mu$ M FITC-conjugated cAMP to the intracellular side of the membrane patch. Application of short laser pulses abolished spHCN inactivation. The channel opening after the laser pulses can be fitted with a single-exponential function, with a time constant around 150msec. After 4-6 laser pulses (100 msec long), the macroscopic current reaches about 30% of maximal current, as determined in the presence of 10 $\mu$ M cAMP. Furthermore, we demonstrate that single point mutation from Histidine to Alanine (H462, located near the intracellular end of S6) eliminates the effects of laser modification. To further investigate the mechanism, we applied 100nM Rose Bengal, a widely used as an organic singlet oxygen generator and observed similar changes to channel activity which suggests that this is photodynamic transformation process that involves a photosensitizer and most likely the generation of singlet oxygen. Thus, together with our previous observation, with the photodynamic transformation of mouse HCN2 channel, we demonstrate that this photodynamic process could be applied to the invertebrate form of HCN channel. The reason for HCN channels to be highly sensitive to photodynamic transformation, most likely through generation of singlet oxygen and the corresponding physiological relevance are open question needed to be addressed in the future study.

#### 1456-Pos Board B407

##### Studying the Influence of the Subunit Arrangement on the Function of Heterotetrameric Olfactory CNG Channels

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Institute of Physiology II, Friedrich Schiller University Jena, Jena, Germany. Cyclic nucleotide-gated (CNG) ion channels, key components in the sensory transduction of vision and odour, are non-selective cation channels, activated by the binding of cAMP or cGMP. Native CNG channels of olfactory sensory neurons are heterotetramers formed by the homologous CNGA2, CNGA4 and CNGB1b subunits in a 2:1:1 stoichiometry. It is still an unsolved question how the individual subunits are arranged with respect to each other.

Addressing this, we used a concatenation approach to force the heterotetrameric channels to a defined order of the subunits. We designed all twelve possible concatamers representing three possibilities of arrangement for the monomers around a central pore. Ligand-dependent activation induced by the binding of either cAMP or cGMP was characterized electrophysiologically for all concatamers in macropatches of *Xenopus laevis* oocytes as well as on the single-channel level. All twelve concatamers formed functional channels with robust expression levels comparable to monomeric channels. As for channels built of monomers, Hill coefficients for cGMP exceeded those for cAMP (1.8 versus 1.5), but were equal for the same ligand with all concatamers. The  $EC_{50}$  values were moderately different among the concatamers without revealing obvious systematics. B1bA2A4A2 proved to be the most and A2A2B1bA4 the least ligand-sensitive channel. Like for channels built of monomers, all twelve concatamers exhibited a typical and complex single-channel behavior with a main single-channel conductance of about 12 pS and 15 pS at positive and negative voltages, respectively.

We conclude that all possible arrangements of the three types of subunits lead to functional heterotetrameric channels with similar properties, suggesting that essential functional specificities inferred by the individual subunits do not critically depend on the nature of their specific neighbor subunits.

#### 1457-Pos Board B408

##### Effect of Ligand Binding to the B1B Subunit of Olfactory CNG Channels

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Institute of Physiology II, University Hospital Jena, Jena, Germany. Cyclic nucleotide-gated (CNG) channels play an essential role in the sensory transduction of the olfactory and visual system. Native olfactory CNG channels are heterotetramers composed of three different subunits: two CNGA2, one CNGA4 and one CNGB1b. It is well established that the presence of the CNGA4 and CNGB1b subunit in heterotetrameric channels leads to an increased apparent affinity for cyclic nucleotides. We have recently shown that the CNGA4 subunit binds cGMP and actively participates to channel activation. Nevertheless, the functional contribution of the CNGB1b subunit is still unclear. By combining mutagenesis and confocal patch-clamp fluorometry we characterized the role of the CNGB1b subunit during channel activation. We show that in contrast to the CNGA4 subunit, that needs only neighbouring CNGA2 subunits for ligand binding, the CNGB1b needs both, CNGA2 and CNGA4. When coexpressing CNGB1b with disabled CNGA2 and CNGA4, channel activation induced by the CNGB1b subunit alone was only weak compared to CNGA4 coexpressed with disabled CNGA2. The apparent affinity ( $EC_{50}$ ) of the CNGB1b subunit under the same conditions is 3.2  $\mu$ M. This is twofold higher than that determined for CNGA4 and 15 fold higher than that of one CNGA2. The binding of cAMP and cGMP to the CNGB1b subunit turned out to be non-selective. Moreover, the kinetics of ligand binding and unbinding as well as channel activation and deactivation for each of the three different subunits in the respective environment of disabled subunits, substantiate the role of the CNGB1b and CNGA4 in speeding up channel closure. In conclusion, we show that olfactory CNG channels are activated by ligand binding to all four subunits, but the contribution of the CNGB1b to channel activation is only modest in comparison with that of the other subunits.

#### 1458-Pos Board B409

##### Explorations of Lipid Effects in Cyclic Nucleotide-Gated Ion Channels using a Nanodisc Platform

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The lipid membranes of cells are complex and dynamic mosaic environments that can alter the activity of membrane proteins including cyclic