$0.21 \pm 0.05$ and $0.13 \pm 0.03$ respectively. At negative voltages divalent cations blocked the inward $Na^+$ current more powerfully in homomeric than in heteromeric channels. These results show that divalent cations permeate more easily through heteromeric than homomeric CNG channels and that they block less powerfully the current carried by monovalent cations in hetoromeric channels.

**562-Pos Board B362**

**Exploring the Response of Bacterial Cyclic Nucleotide Gated (bCNG) Ion Channels to Mechanical Stress**

**Hannah R. Malcolm**, Donald E. Elmore, Joshua A. Maurer.

The bacterial cyclic nucleotide gated (bCNG) ion channel family contains a N-terminal channel domain, which is homologous to the Mechanosensitive Channel of Small Conductance (MscS) and a ligand binding domain with sequence similarity to known cyclic nucleotide binding domains. Previously, we have demonstrated that some of these channels gate in response to cyclic adenosine monophosphate alone [Caldwell, et al, BBA 2010, 1798, 1750-1756]. Here we explore the ability of bCNG channels to gate in response to mechanical stress, which one might predict based on their significant sequence homology to MscS. To this end, we measured the ability of fourteen distinct bCNG channels from several different bacterial strains to rescue E. coli lacking mechanosensitive channels (MscS/MscL/MscK null) from osmotic downshock. In our studies, only two bCNG channels exhibit limited ability to rescue bacteria from osmotic downshock. These two channels were found in bacterial strains with genomes encoding for multiple variants of the bCNG gene. Only one bCNG channel variant in each strain was capable of rescuing E.coli from osmotic downshock, implying that each gene product may have a unique role or that the two gene products may work in concert. Additionally, truncation of the cyclic nucleotide binding domain in some non-mechanosensitive variants of bCNG increases their response to mechanical stress.

**563-Pos Board B363**

**Analysis and Minimization of Ligand Concentration Errors at the Internal Face of Excised Patches**

**Jana Kusch**, Ralf Schmauder, Christoph Biskup, Vasilica Nache, Klaus Benndorf.

Confocal patch-clamp fluorometry is a method combining patch-clamp techniques and confocal fluorescence microscopy for simultaneous investigation of channel activation and ligand binding in ion channels, as used for CNGA2 olfactory channels and HCN2 pacemaker channels. The fluorescent ligands fcAMP and fcGMP, and the reference DY647, used in these studies were negatively charged. Their fluorescence signals in the volume between patch membrane and pipette tip can depend significantly on the applied voltage if the conditions are not optimized, indicating a difference between actual ligand concentration at the patch and in the bath.

Using confocal PCF and fluorescence correlation spectroscopy, we analyzed this phenomenon and looked for conditions to overcome this problem. (1) When investigating not or only slightly voltage dependent ion channels, we propose to use low voltages. (2) When investigating ion channels with a high single channel conductance, causing macroscopic ion currents in the range of tens of nA, we propose a reduced expression to keep the membrane resistance reasonably high. (3) For all ion channel types we recommend the use of pipette tips with a planar surface following the fabrication protocol of Hilgemann (1995). These pipette shapes avoid any significant additional voltage drop across the pipette tip and thus also the described errors of the charged molecules.

We compare the activation and binding behaviour of CNG channels, having a minor voltage dependence and a high single channel conductance, with closely related HCN channels, having a pronounced voltage dependence and a low single channel conductance. It is concluded that in excised macropatches with large currents the concentration of charged molecules at the internal face of the patch can significantly deviate from that in the bath solution and that appropriate experimental conditions can largely avoid the problem.

**564-Pos Board B364**

**A D262n Mutation in Canine CNGB3 in a Highly Conserved Acidic S2 Region Causes Complete Colorblindness in a Canine Model**

**Naoto Tanaka**, Billy J. Pérez, Ashley Schloss, Armando Rosario, Asli Kaya, Andras M. Komaromy, Jacqueline C. Tanaka.

Cyclic nucleotide-gated (CNG) channels in cone photoreceptors are composed of CNGA3 (A3) and CNGB3 (B3) subunits. In a canine achromatopsia model, loss of cone ERG function and color vision are associated with the mutation of Asp (D) 262 to Asn (N) in the B3 subunit. This Asp residue is conserved in all CNG channels. We examined the D/N mutation in the human (h) B3 channel co-expressed with a wild-type canine (c) A3 and in a mutant cA3 homomeric channel in which the conserved Asp residue was substituted with Asn. In patch clamp experiments, both heteromeric wild type cA3 co-expressed with D/N hB3 and mutant homomeric D/N cA3 CNG channels had no cGMP-activated currents providing molecular insights into the cause of canine achromatopsia. D262 of the cB3 is located in the S2 transmembrane domain and, along with D252 and D256, these residues comprise an acidic motif conserved in the CNG channel family and extending to some members of the Shaker K superfamily. Hints about the role of this Asp motif have been gained from studies of related channels reporting interactions between the negatively-charged residues in S2 and the conserved positively-charged residues in other transmembrane domains. We generated mutant A3 subunits by substituting each of the three conserved S2 Asp residues with Asn, Cys, or Glu. Patch clamp analysis showed all of the mutant homomeric A3 channels lost function. These YFP-tagged A3 mutant channels exhibited changes in cellular localization compared to wild type channels suggesting that any change in the Asp motif alters subunit trafficking. Future studies will focus on understanding the role of this Asp motif in intra-subunit helical contacts and proper trafficking and assembly of cone CNG channels.

**565-Pos Board B365**

**Mutations in the Canine CNGB3 Channel Subunit in Two Achromatopsia Models Leads to Mis-Localization of CNGA3 Expression in Retinal Outer Segments**

**Amaliris Gonzalez**, Asli Kaya, Jessica S. Rowlan, Andras M. Komaromy, Jacqueline C. Tanaka.

Cone cyclic nucleotide-gated (CNG) channels, composed of CNGA3 and CNGB3 subunits, play a central role in color vision. Along with other genes, mutations in CNGB3 are associated with achromatopsia, an inherited autosomal recessive disorder that results in loss of color vision and cone function. Two independent canine colonies were bred with mutations in CNGB3; the affected dogs lacked cone ERG function. One mutant colony has a missense mutation at residue 262 with Asp replaced by Asn, denoted CNGB3m/m, and the other colony has a deletion resulting in loss of the CNGB3 gene, denoted CNGB3-/- . We examined expression of cone CNG channels in the retinas of carrier (CNGB3m/+ or CNGB3-/+) and affected dogs using immunohistochemistry and immunoblotting with an anti-canine CNGA3 antibody, qRT-PCR and in-situ hybridization. qRT-PCR shows that missense-CNGB3 mRNA is expressed in retinal extracts of CNGB3m/m dogs but not in CNGB3-/- dogs. Of Interest, retinas from all affected dogs show complete loss of CNGA3 immunoreactivity in the outer segments despite evidence that both the CNGA3 mRNA and the protein are expressed in normal and affected retinas. Affected dogs were treated with rAAV-mediated gene replacement therapy with the human CNGB3 transgene using a human red cone opsin promoter. A single subretinal injection in one eye was sufficient to restore cone ERG function in a majority of treated dogs. Examination of CNGA3-immunoreactivity in the retinas of treated dogs showed that the human CNGB3 transgene directed CNGA3 expression to the cone outer segment. Future studies will monitor CNG channel subunit composition directly to address whether the Asp262Asn mutation can participate in CNG channel formation.

**566-Pos Board B366**

**Structural and Biochemical Study of CAMP-Dependent Regulation of Human HCN4 Channel**

Xinping Xu, Zhanna V. Vysotskaya, Qinglian Liu, **Lei Zhou**.

Hyperpolarization-activated cAMP-regulated (HCN) channels have important functions in both cardiovascular and central nervous systems. HCN family contains four HCN isoforms of high similarity in primary amino acid sequence. Here we studied human HCN4 channel, the major form expressed in heart, by a combination of electrophysiology, structure biology and biochemistry approaches. Comparing with mouse HCN2, we observed the hHCN4 protein exhibited marked different contributions to channel function, such as an approximately 3-fold reduction in the response to cAMP. Moreover, upon cAMP binding, hHCN4 C-terminal protein prolongs channel deactivation more effectively, which could results in increased net currents during the diastolic depolarization and thus significant physiological contributions. To figure out the corresponding structural basis, we solved the crystal structure of the hHCN4 channel C-terminal fragment at 2.4Å. hHCN4 structure is very similar to the previously published mHCN2 structure, except a loop region between two beta-strands. Guided by the structural differences, we identified residues that could be partially accounted for the differences in the response to cAMP between mHCN2 and hHCN4 proteins. Moreover, to separate the issues of binding affinity and gating efficacy, we applied two biochemical methods, isothermal titration calorimetry and fluorescence anisotropy, to measure the binding affinity of cAMP to purified HCN protein samples. Our results show that the binding affinity (Kd) is about 10-30 times higher than the value of apparent affinity (K1/2) or half effective concentration (EC50) for cAMP to shift the voltage-dependent gating curve.