functional homotetrameric channels that can be activated in a cooperative manner by cAMP or cGMP binding to the cyclic nucleotide-binding domains (CNBD) included in each subunit. Our aim was to kinetically further dissect the molecular mechanism leading to channel activation upon ligand binding and to channel deactivation upon ligand removal.

CNGA2 channels, expressed in Xenopus oocytes, were studied in excised patches by measuring simultaneously ligand binding/unbinding and activation/deactivation by means of confocal patch-clamp fluorometry under steady-state and non-steady state conditions (182 or 277 frames per second). Concentration jumps of a fluorescent cGMP derivative (Biskup et al., Nature, 446(7134): 440-3, 2007) were applied using a fast piezoelectric system. Surprisingly, the binding was concentration dependent while deactivation was concentration independent. The unbinding was approximately 100 times faster from fully liganded channels in comparison with the unbinding from lowly liganded channels. The obtained data were analyzed by global fits to various types of Markovian state models. The additional information of unbinding and deactivation allowed us to refine the previously determined C4L-Model (Biskup et al., Nature, 446(7134): 440-3, 2007). To account for the very fast unbinding at saturating ligand concentrations, the C4L-Model had to be expanded: When fully liganded, the channel adopts an open state which allows, upon ligand removal, a very fast unbinding of all four ligands. In contrast, from partially liganded states this fast unbinding is occluded. Our results suggest an additional pathway for rapid ligand unbinding for the fully liganded channel.

1439-Pos Board B331
Probability Fluxes and Transition Paths in a Markovian Model Describing Complex Subunit Cooperativity in HCN2 Channels
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Hyperpolarization-activated cyclic nucleotide-modulated (HCN) ion channels are voltage-gated tetrameric cation channels that generate pacemaker activity in neurons and cardiomyocytes. Activation of these channels can be enhanced by the binding of adenosine-3’,5’-cyclic monophosphate (cAMP) to an intracellular cyclic-nucleotide binding domain in each of the four subunits. Based on previously determined rate constants for a complex Markovian model describing the gating of homotetrameric HCN2 channels (Kusch et al., Nat. Chem. Biol. 8, 162-9, 2012), we analyzed probability fluxes within this model, including unidirectional probability fluxes. Following the rules of the transition path theory, we analyzed the transition paths in our model for channel activation, following a jump to a defined ligand concentration from zero, and for channel deactivation, following a jump from the ligand concentration back to zero. Three ligand concentrations were considered. The time-dependent probability fluxes quantify the contributions of all 13 transitions of the model to channel activation. The binding of the first, third and fourth ligand evoked robust channel opening whereas the binding of the second ligand obstructed channel opening similar to the empty channel. Our analysis of the net probability fluxes revealed the pronounced hysteresis for channel activation and deactivation.

These results provide insight into the complex cooperative interaction of the four subunits equal by sequence, leading to pronounced differences in the subunit function.

1440-Pos Board B332
A Canine CNGB3 Channelopathy Suggests that Changes in Calcium Homeostasis Result in Progressive Loss of Cone Function
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Canine day-blindness, a model for human achromatopsia, is associated with loss of cone function due either to the deletion or a missense Asp (D) 262 to Asn (N) mutation in CNGB3. Asp 262 resides in an acidic motif in the S2 transmembrane helix conserved in all CNG channel subunits and members of the Shaker K+ superfamily. Tetrameric cyclic nucleotide-gated (CNG) channels are formed from CNGA3 and CNGB3 subunits and transduce light information in cone photoreceptor outer segments. In canine day-blindness, the CNGB3-D262N mutation leads to loss of cone function between 4 and ~10 weeks suggesting progressive physiological changes. We investigated the missense mutation using the human CNGB3, previously used in gene therapy to restore cone function in young dogs (Hum Mol Genet 2010 19: 2581). Canine CNGA3 was co-expressed with hCNGB3; the most significant functional difference between homomeric and heteromeric currents was an ~10 fold increase in PCa/PNA in heteromeric channels. Co-expression of cCNGA3 with hCNGB3-D262N (canine numbering) result in the absence of functional heteromeric channels with evidence of some homomeric CNGA3 channels. We suggest that alterations in calcium homeostasis associated with the missense mutation in CNGB3 contribute to the loss of cone function. We generated mutations in the Asp residues in CNGA3 channels. We investigated substitutions in the three Asp residues in S2 and all mutations examined resulted in the loss of channel function underscoring the essential role for these residues in channel function.

Studies in voltage-gated channels show electrostatic interactions between the acidic residues in S2 and residues in S3 and S4 transmembrane domains. Our future experiments will explore the role of these acidic residues in intrasubunit helical interactions using mutagenesis and molecular modeling.

Cardiac, Smooth, & Skeletal Muscle Electrophysiology I

1442-Pos Board B334
Endogenous VIP May Contribute to Vagal Induced Electrophysiological Changes in Canine Atria
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Background: There has been increasing evidence that complex interactions among the various components of intracardiac neural network play an important role in atrial fibrillation (AF). Perfusion of vasoactive intestine polypeptide (VIP), a neural polypeptide, co-released with acetylcholine from intrinsic cardiac neurons during vagal stimulation, was shown to shorten the action potential duration (APD), decrease the intraatrial conduction velocity (CV), and promote induction of AF. However, the effect of endogenous VIP remains unclear.

Methods: In 6 isolated arterially perfused canine left atria, high-resolution optical mapping techniques with di-4-ANEPPPS and blebbistatin were used to measure APD and CV during fat-pad ganglion plexus stimulation (GPS, 30Hz, 10.2 ± 2.3Volt validated with blockage of atrioventricular conduc- tion), at during H9335, a VIP antagonist (1 5 8ms at baseline and 125 5 8ms, p < 0.05; CV: 105 5 10ms after recovery, p < 0.05), which recovered within 2 min (APD: 128 5 8ms, p < 0.05; CV: 105 5 13cm/sec, p < 0.05). With H9335, the APD shortening effect (17%) of GPS persisted (GPS, 105 14ms, vs. 127 5 8ms at baseline and 125 5 10ms after recovery, p < 0.05) with a trend towards being less pronounced as compared to GPS effect without H9335.