determined (Michels et al., Circulation, 2005). Later Dekker and Yellen (J. Gen. Physiol., 2006) confirmed a small conductance of ~1.5 pS but observed a pronounced cooperativity of multiple channels. We expressed HCN2 channels in Xenopus oocytes and studied single channel currents in inside-out patches. Our results confirm a small conductance (~2 pS) and do not provide any evidence for a cooperative gating between the channels, enabling recording from patches containing one and only one channel. The activating effect of cAMP, applied to the bath solution, is mediated by an increase of the open probability. In conclusion, HCN2 channels expressed in Xenopus oocytes develop an only small single-channel conductance, gate as individual channels, and are activated by cAMP via an increase of the open probability. These results are of importance for modelling single-channel properties from macroscopic currents.

1434-Pos Board B326
Voltage- and CaM-Dependent Gating in Heterotetrameric HCN2/4-Pacemaker Channels
Jana Kusch, Jana Rose, Tobias Fischer, Susanne Thon, Klaus Benndorf.
Friedrich-Schiller-University, Jena, Germany.
HCN pacemaker channels play an important role in generating and regulating rhythmicity of special neurons and cardiac cells. They are activated by hyperpolarizing voltages and modulated by the binding of cyclic nucleotides. Four isoforms, HCN1-4, have been identified. HCN2 and HCN4, expressed in cardiac sinoatrial node and ventricular cells, build functional homotetrameric and heterotetrameric channels in various heterologous cell systems. Heterotetrameric HCN2/4 channels in Xenopus oocytes show two changes compared to each of the homotetramers: the voltage of half maximum activation is shifted to more depolarized voltages and activation kinetics are faster (Zhang et al., Biochim Biophys Acta, 2009). However, little is known about the ligand dependence of these channels. Herein, we studied the differences in activation gating of HCN2/4 channels in comparison to the respective homotetramers, thereby focusing on the effect of CaM. We monitored activation under both steady-state and non steady-state conditions in the presence and absence of CaM, as well as the ligand-dependent activation kinetics after ligand jumps.

We found (1) that in HCN2/4 the apparent affinity for cAMP was between that of the two homotetramers, whereas the Hill coefficient was lowest, (2) that cAMP accelerates voltage-induced activation in HCN2/4 only slightly (factor ~2), resembling HCN4, whereas in HCN2 it accelerates activation in a voltage-dependent manner by a factor of up to ~12, (3) that the activation kinetics following a cAMP jump to channels pre-activated by voltage was fastest in HCN2/4, whereas the increase of current amplitude by a concentration jump following a cAMP jump to channels pre-activated by voltage was fastest in HCN4 expressed in Xenopus oocytes. But when the formation of heterotetramers is age-dependent by a factor of up to ~12, (3) that the activation kinetics jumps.

The ligand dependence of these channels. Herein, we studied the differences in activation gating of HCN2/4 channels in comparison to the respective homotetramers, thereby focusing on the effect of cAMP. We monitored activation under both steady-state and non steady-state conditions in the presence and absence of CaM, as well as the ligand-dependent activation kinetics after ligand jumps.

We found (1) that in HCN2/4 the apparent affinity for cAMP was between that of the two homotetramers, whereas the Hill coefficient was lowest, (2) that cAMP accelerates voltage-induced activation in HCN2/4 only slightly (factor ~2), resembling HCN4, whereas in HCN2 it accelerates activation in a voltage-dependent manner by a factor of up to ~12, (3) that the activation kinetics following a cAMP jump to channels pre-activated by voltage was fastest in HCN2/4, whereas the increase of current amplitude by a concentration jump following a cAMP jump to channels pre-activated by voltage was fastest in HCN4 expressed in Xenopus oocytes. But when the formation of heterotetramers is age-dependent by a factor of up to ~12, (3) that the activation kinetics jumps.

1435-Pos Board B327
Voltage Gated Cation Channels Activation: Towards an Ab-Initio Kinetic Model
Lucie Delemotte1, Marina Kasimova2, Mounir Tarek2, Werner Treptow3, Vincenzo Carnevale4, Michael L. Klein1.
1Temple University, Philadelphia, PA, USA, 2Université de Lorraine, Nancy, France, 3Universidade de Brasília, Brasília, Brazil.
Many modulators, such as toxins, anesthetics or drugs, act on voltage-gated ion channels (VGCCs) by altering the kinetics of activation and/or deactivation of their voltage-sensing domains (VSD). So far, we have proposed a “static” model of the Kv1.2 VSD activation using brute force and modified molecular dynamics simulations and that agrees with a large body of experimental data. This model involves 5 states: α (activated), β, γ, δ (three intermediate) and ε (resting) [Delemotte et al. 2011, Proc. Natl. Acad. Sci. USA, 108:6109-6114], and has enabled to gain access to the contribution of transmembrane voltage to the free energy of activation via calculation of the corresponding gating charge. Crucial details, however, are still missing, among which an estimation of the thermodynamic and kinetic stability of these states or of the minimum energy transition pathway linking them.

In order to complete our understanding of VSD function, we produce the free energy landscape (FEL) of the four transition states of the Kv1.2 VSD conformations and estimate therefore the corresponding the rate (kinetic) constants. This enables, not only to follow for the first time the pathway of activation of a VGCC VSD, but also to produce a complete ab-initio kinetic model of its activation based uniquely on parameters derived from an in-silico investigation. The G/V and Q/V curves (ionic current and gating current/voltage, respectively) characteristic of the function of wild type channels derived therefrom are then compared to electrophysiology recordings. The study is then extendable to investigate the modulation of VSD function by drugs, toxins, mutations or else lipid interaction.

1436-Pos Board B328
Acidic pH Uncovers Desensitization and Structurally-Distinct Types of Voltage Gating in CNGA1 Channels
Manuel Arracheletti1, Arin Marchesi1, Monica Mazzolini1, Vincent Torre1,2, Stephen Altieri1, Lise Thomas2, João H. Morais-Cabral1.
1IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, 2ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal.
Cyclic nucleotide-gated (CNG) channels are members of the superfamily of voltage gated ion channels, but in the presence Na+ and K+ they are gated primarily by cyclic nucleotides (CNs) and only weakly by voltage. Here, we show that when extracellular pH (pHo) is decreased from pH 7.4 to 5 WT CNGA1 channels desensitize, i.e. the current activated by a steady cGMP concentration decreases within 5-40 s by 20-80% in a voltage dependent way. Current desensitization is completely reversible upon removal of cGMP and voltage dependency of desensitization is associated to the displacement of 0.3 equivalent electronic charges across the electrical field. A very similar desensitization is observed in several mutant channels, such as E363A and T364A at the usual pH 7.4. At the desensitized state, the IV relations are outwardly rectifying similarly in the WT CNGA1 channels at pH 5 and mutant channels E363A at pH 7.4. In the presence of symmetrical Rh+ or Cs+, the single channel conductance gsc in WT CNGA1 channels is highly voltage dependent and this voltage dependence is abolished in mutant channels E363A and T364A. CNGA1 channels have structurally-distinct types of voltage sensors, formed by charged and polar residues located in the pore, by the usual S4 voltage sensor and by an additional voltage sensor associated to the voltage dependency of desensitization. Our results not only shed a new light on voltage gating in CNG channels, but uncover the existence of a novel component of phototransduction: neuronal signalling within the vertebrate retina is highly dependent on the pHo and proton elevation could act as a negative feedback.

1437-Pos Board B329
Exploring the Mechanism of Cyclic Nucleotide Activation in the MLOTIK1 Potassium Channel
João Pesoa1,2, Stephen Altieri1, Lise Thomas2, João H. Morais-Cabral1.
1IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, 2ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal.
Cyclic nucleotides are essential elements in the cellular response to hormones, light and smell. These molecules bind to their receptors through a well conserved cyclic nucleotide binding (CNB) domain, propagating a conformational change to an effector domain. We seek to understand the molecular details of the cyclic nucleotide activation mechanism. Our model protein is the CNB domain of a cyclic nucleotide regulated potassium channel from Mesorhizobium loti, a soil bacterium. This CNB domain is formed by a β-roll and three α-helices, named αA, αB and αC. A major difference between the bound and unbound states is the relative position of αC helix, which raises the hypothesis that αC helix motion could be the primary event in cyclic nucleotide binding.

To address the hypothesis, we have monitored the conformational change in the isolated CNB domain. We tested two mutants having a single cysteine in αB or αC helix using a cysteine-reacting probe. Reaction kinetics were quantified by determining rate constants, which reflect the relative exposure of the cysteine. The cyclic nucleotides tested were not all equivalent in their rate constants. Moreover, the ratios between rate constants determined in the same conditions were different for αB and αC helix mutants. This indicates that ligand binding does not have the same effect on the two connected helices or, in other words, that the conformational change in αB helix is not totally dependent on that in αC helix. Therefore, the primary event in ligand binding is not the αC helix motion. Accordingly, point mutations in a functionally relevant residue in αC helix affected activity of the full-length channel only partially, suggesting that residues outside this helix must be involved in the activation mechanism.

1438-Pos Board B330
Deactivation of CNGA2 Channels follows Intricate Pathways
Vasilica Nache1, Eckhard Schulz2, Thomas Eick1, Klaus Benndorf1.
1University Hospital Jena, Jena, Germany, 2University of Applied Sciences, Schmalkalden, Germany.
Cyclic nucleotide-gated (CNG) channels in olfactory neurons are heterotetrameric ligand-gated cation channels that are composed of four homologue subunits (2xCNGA2, 1xCNGA4, 1xCNGB1b). Only the CNGA2 subunits form acidic pH Uncovers Desensitization and Structurally-Distinct Types of Voltage Gating in CNGA1 Channels
functional homotetrameric channels that can be activated in a cooperative manner by cAMP or cGMP binding to the cyclic nucleotide-binding domains (CNRB) 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 unbinding 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
Klaus Benndorf1, Jana Kusch1, Eckhard Schulz2

1Friedrich-Schiller-University, Jena, Germany, 2University of Applied Sciences, Schmalkalden, Germany.

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 intraacellular 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 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
Naoto Tanaka1, Amaliris Gonzalez1, András M. Komaromy2, Jacqueline C. Tanaka1

1Temple University, Philadelphia, PA, USA, 2Michigan State University, East Lansing, MI, USA.

Canine day blindness, a model for human achromatopsia, is associated with loss of cone function due 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 IpcA/PNA, a functional channel 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 intra-subunit helical interactions using mutagenesis and molecular modeling.

Cardiac, Smooth, & Skeletal Muscle Electrophysiology I

1442-Pos Board B334
Endogenous VIP May Contribute to Vasculally Induced Electrophysiological Changes in Canine Atria
Yutoa Xi1,2, Bao Pham1, Junping Sun1, Geru Wu1, Steven Lee1, Cai Chen1, Wen Yan1, Zhi-Yang James Chao1, Shahzad Abbas1, Jie Cheng1,2

1Texas Heart Institute, Houston, TX, USA, 2University of Texas School of Medicine at Houston, Houston, TX, USA.

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 intestinal 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-ANEPPS 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 conduction), at during H9335, a VIP antagonist (1M) was used to block the sympathetic pathway. Averaged APD was shorter (21%) during GPS compared to the baseline (100±8ms vs. 126±7ms, p<0.05), and average CV was slower than baseline (87±10cm/sec vs. 103±13cm/sec, p<0.05), which recovered within 2 min (APD: 128±8ms, p<0.05; CV: 105±13cm/sec, p<0.05). With H9335, the APD shortening effect (17%) of GPS persisted (GPS, 105±14ms, vs. 127±8ms at baseline and 125±10ms after recovery, p<0.05) with a trend towards being less pronounced as compared to GPS effect without H9335.