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 cooperative gating 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.

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Voltage- and Camp-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-HCN4, 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 cAMP. We monitored activation under both steady-state and non steady-state conditions in the presence and absence of cAMP, 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 was similar to homotetramers. Our results confirm that coinjection of the HCN2 and HCN4 isoforms in *Xenopus* oocytes leads to heterotetrameric channels. They suggest that in native heart cells the formation of heterotetramers leads to pacemaker channels with specific characteristics, thereby fine-tuning the process of pacemaking.

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Voltage Gated Cation Channels Activation: Towards an Ab-Initio Kinetic Model

Lucie Delemotte[1], Marina Kasimova[2], Mounir Tarek[2], Werner Treptow[3], Vincenzo Carnevale[1], Michael L. Klein[1].
[1]Temple University, Philadelphia, PA, USA, [2]Université de Lorraine, Nancy, France, [3]Universidade de Brasília, Brasília, Brazil.

Many modulators, such as toxins, anesthetics or drugs, act on voltage-gated cation 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 (FES) of the four transitions linking the Kv1.2 VSD conformations and estimate therefrom 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.

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Acidic pH Uncovers Desensitization and Structurally-Distinct Types of Voltage Gating in CNGA1 Channels

Manuel Arcangeletti[1], Arin Marchesi[1], Monica Mazzolini[2], Vincent Torre[1].
[1]SISSA, Trieste, Italy, [2]CBM, Trieste, Italy.

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 declines 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 I/V 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 Rb+ 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 phototrasduction: neuronal signalling within the vertebrate retina is highly dependent on the pHo and proton elevation could act as a negative feedback.

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Exploring the Mechanism of Cyclic Nucleotide Activation in the MLOTIK1 Potassium Channel

João Pesoa[1,2], Stephen Altieri[3], Lise Thomas[4], João H. Morais-Cabral[1].
[1]IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, [2]ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal, [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [4]Department of Biological Sciences, Quinnipiac University, Hamden, CT, USA.

Cyclic nucleotides are essential elements in the cellular responses 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.

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Deactivation of CNGA2 Channels follows Intricate Pathways

Vasilica Nache[1], Eckhard Schulz[2], Thomas Eick[1], Klaus Benndorf[1].
[1]University Hospital Jena, Jena, Germany, [2]University 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