leucyl-L-norleucinal and MG132 prevented the 5α-DHT-dependent enhancement of HERG as did the lysosome inhibitor, bafilomycin A1. Consistently, the cycloheximide-based protein chase study showed that 5α-DHT prolonged HERG protein half-life. 5α-DHT/AR45 signaling induced phosphorylation of extracellular signaling regulated kinase (ERK1/2). Blockade of ERK1/2 with PD98059 and U0126 prevented the effect of androgen on HERG protein abundance. Functional studies showed that 5-DHT treatment for 24 h increased HERG K^+ current density in CHO cells co-transfected with cDNAs of AR45 and HERG channels. Moreover, 5α-DHT also increased ERG protein abundance in isolated rabbit cardiac myocytes. In conclusion, these data provide evidence that stimulation of AR45 receptors by androgens upregulates HERG K^+ channel abundance and activity mainly through stabilizing HERG protein in an ERK1/2 dependent mechanism and suggest a mechanism to explain the sex difference in the long QT syndrome.

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Insights into the Ion Selectivity Mechanism of CNG Channels from Mutants of NaK: Structural and Functional Studies

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Cyclic nucleotide-gated (CNG) channels are non-selective cation channels that play crucial roles in visual and olfactory signal transduction. They are members of the tetrameric cation channel family that include voltage-gated K^+, Na^+ and Ca^{2+} channels. However, while other members exhibit high degree of ion selectivity, CNG channels are noted for their lack of specificity. CNG channels conduct all alkali metal ions and some alkaline earths, most notably Ca^{2+}. How the CNG channel pore can conduct these various cations which have substantially different ionic radii and formal charges is not well understood. Here we report high-resolution crystal structures of mutants of the NaK channel that mimic the selectivity filter of CNG channels, along with supporting functional analyses. Within the NaK selectivity filter (^{63}TVGDGNFS^{70}) the DGNFS sequence was replaced with ETPP, ETPT and DTPS, each of which represent a CNG α-subunit sequence. The mutant structures exhibit selectivity filter architecture and ion binding profiles different from either NaK or K^+ channel structures, having three ion binding sites in their selectivity filters. Two of the sites correspond to sites 3 and 4 in KcsA and NaK, while the third site corresponds to site 2 in KcsA, but is a vestibule in NaK. Similar to CNG channels these mutants exhibit calcium binding, which depends on the presence of the conserved acidic residues (E or D). Mutating the acidic residues on these mutants to neutral residues (E◇Q or D◇N) abolishes calcium binding. Functional analyses using Rb-86 flux assay revealed ion conduction behavior similar to CNG channels. These results provide strong evidence that these NaK mutants exhibit the same properties of CNG channels in ion conduction and selectivity and their structures provide insight into understanding ion selectivity in CNG channels.

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Water Dominated Ions Stability and Conduction in NaK Channel

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Water plays an important role in ion channels. It stabilizes ions in the central cavity and accompanies them to permeate through the channel, and it also participates in processes of ion selection. Here we find four water grottos connecting with the vestibule of the NaK selectivity filter, and they form a vestibule-grotto (V-G) complex in a plane perpendicular to the ion conducting pore. Molecular dynamics (MD) simulations show that water can penetrate and escape the grottos from the extracellular water pits above the grottos around the extracellular entrance, and two aromatic residues Tyr55 and Phe56 serve as a gate between the grottos and water pits. In the rest state, water molecules are confined in the vestibule and grottos and seldom exchange between them, and they have little impact on the K^+ ion binding states in the selectivity filter. While in the active state, the water molecules in the V-G complex become highly activated and they can flow easily between the vestibule and grottos. MD and free energy calculations show that the water molecules moving in the V-G complex hydrate and stabilize ions in the filter and serve as a valve in conveying ions through the vestibule for controllable ion permeating. The existence of the grottos and the simple and beautiful structure-function correlation of the hydration valve can be expected in the whole family of CNG channels, which function in our photoreceptors and olfactory cells.

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Functional investigation of the light-gated Channelrhodopsin

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The retinal proteins Channelrhodopsin-1 and −2 (ChR-1 and −2) from *Chlamydomonas reinhardtii*, which were first described as light-gated ion channels by Nagel *et al.* in 2002 and 2003, emerged in the last few years as advantageous tools. Since they open up rapidly after absorption of a photon and permeate ions like sodium or calcium, Channelrhodopsins are already used for non-invasive excitation of excitable cells in culture as well as in living tissue.
Together with other retinal proteins they share a 7-transmembrane helix motif where the retinal chromophore is covalently linked to the protein via a protonated Schiff base. Recent investigations by Bamann *et al.* (2008) predicted a photocycle with at least 4 photointermediates, all coupled to the channel function. But little is known about the mechanism that infers the properties of the ion channel or the channel pore, especially the different permeability coefficients between a series of cations and the strong inward-rectifying behaviour of the photocurrents is not fully understood.
Here we present a detailed functional characterisation by patch-clamp measurements on HEK293 cells stably expressing Channelrhodopsins. We could show that the inward rectifying properties are associated with the availability of cations and therefore predict cation binding to the protein. These results are discussed in relation to the hypothetical structure of the Channelrhodopsin and a putative cation binding site.

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Mutations in Cys 128 cause extreme decelerations of the Channelrhodopsin-2 kinetics

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Channelrhodopsin-2 (ChR2) triggers the phototaxis of the green algae *Chlamydomonas reinhardtii*. Amino acids 1-315 form a Bacteriorhodopsin (BR)-like heptahelical membrane domaine, which comprises the ion permeability and the covalently bound retinal chromophore. Depending on the electrochemical gradients, protons and cations are conducted in both directions upon light activation of ChR2. However, only little is known about the residues that determine the channel function, while proton pumping BR has characterized in almost any detail. In order to ensure a unidirectional charge transfer, BR goes through a photocycle with separated proton uptake and release in which only one proton is pumped. In contrast, Channelrhodopsins conduct hundreds of ions during each cycle. Hence, we deduce that these proteins are approaching a defined photointermediate which forms an open channel pore, i.e. the conducting state. By Two Electrode Voltage Clamp (TEVC) measurments in *Xenopus* oocytes, we identified an amino acid that plays a crucial role in this process. Mutation of C128 in Helix3 to Thr, Ala or Ser, decelerates the ChR2 kinetics dramatically. For instance, the on-kinetics of C128A is 10 times slower and the off-kinetics even 2000 times slower compared to the wilde type. In addition, we show that cells expressing these mutants are more than 300 times more light-sensitive than ChR2-WT and that they can be used as photo-switches. In BR, a corresponding threonine (BR-Thr90) is located near the 11-12 position of the retinal and is involved in the rearrangement of the α-helices during the photocycle[1]. We conclude that Cys 128 is critical for both fast opening anf fast closing of the of the ion channelpore.

1. Joh, N. H., Min, A., Faham, S., Whitelegge, J. P., Yang, D., Woods, V. L., and Bowie, J. U. (2008) *Nature* **453**, 1266-1270

3466-Pos Board B513

Four Intrinsic Aqueduct Orifices Outstretched from the Central Cavity Facilitate Potassium Channels Gating

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Potassium channels enable K+ ions to flow selectively across cell membrane through a central pore. The mechanisms of ion selectivity and channel gating have long been the attractive secrets. The breakthrough in determination of the structure of the KcsA potassium channel (Science 280, 69-77, 1998) has raised a high tide in structure and function study, but channel gating still remains a long secret. The core structure of K+ channels was found to be highly conserved, and constructed of an inverted teepee with a large water-filled cavity at center and the well studied selectivity filter at its wide end. Here we find four aqueduct orifices outstretched from the cavity and perpendicular to the central pore, leading to shape of a swastika or Greek Fleurée Cross, having subtle gating function. We demonstrated by systematical molecular dynamics simulations that water molecules flowing in the orifices can harmonize the space changing in the cavity to reduce the opening resistance significantly, and blocking the aqueduct orifices makes the intracellular entryway difficult to be opened. This is strongly supported by existed mutation experiments. Homology analyses of all available pore structures and amino acid sequences of K+ channels show that the aqueduct orifices are intrinsic structure feature to the whole potassium channel genre, but their size and conformation are less conserved among different subfamilies, shedding light on their functional diversity.