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.

3462-Pos Board B509

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.

3463-Pos Board B510

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.

3464-Pos Board B511

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.

3465-Pos Board B512

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.

Potassium and water channels have been the wining channels for 2003 Nobel prize. The finding of the intrinsic aqueduct orifices and their vital functions in channel gating shows water flowing merges with ion activity. The orifices even exist in the newly determined atomic structure of NaK channel (Nature 440, 570-574, 2006), which belongs to another large ion channel family (cyclic nucleotide-gated channels).

3467-Pos Board B514

ChannelTools: A VMD Plugin For Manipulating, Visualizing and Measuring Ion Channel Properties

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A new set of software tools has been developed for manipulating, visualizing, and quantifying properties of ion channels. ChannelTools is a plugin for the Visual Molecular Dynamics (VMD) program and brings together native VMD commands and several independent software routines in one simple graphical user interface. ChannelTools allows quick and easy visualization of ion channels and employs the HOLE program to visualize the channel pore and pore lining surface. An option to apply the atomic co-ordinate standardization previously developed by the authors is implemented yielding a consistent channel orientation, axis, and geometric centre. By employing the ESFERA program, pore volume and surface area can be computed from HOLE program output at the click of a button. Cross-sectional area and radius profile data and plots can also be generated with a single button click. Several different routines for estimating channel conductance are also being developed.

3468-Pos Board B515

Assay Development And Screening For Modulators Of The Human Two-pore Domain Potassium Channel, TASK-3, Using Automated Electrophysiology

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The advent of high-throughput electrophysiology utilizing Population Patch Clamp™ (PPC) technology has allowed the screening of large compound libraries against ion channels representing novel targets in a variety of disease states. Here we report the design and implementation of an assay enabling the screening of 56,000 compounds against human TASK-3, a member of the two-P potassium channel family.

cDNA corresponding to the channel of interest was transfected into HEK-293 cells and a stably-expressing clone selected. Ion channel pharmacology was subsequently validated using Ruthenium Red, Lidocaine, Bupivicaine, Quinidine & pH, with all 5 standard inhibitors giving values within two-fold of reported literature values.

The biophysical properties of TASK-3 prevents the use of conventional methods of leak calculation, however, given the relatively low seal resistance routinely seen when using IonWorks® platforms in a PPC™ mode, some form of leak correction must be applied. Currents were recorded at 0mV in order to remove any effect of leak and a final addition of a supramaximal concentration of Ruthenium Red was used in order to calculate a current window from which to base the efficacy of compounds on.

Assays were performed at pH 7 (EC_{75}) in order to screen for potentiators as well as inhibitors. Data derived from the voltage step to 0mV was analysed using Genedata. Inhibitory compounds showing $<25\%$ control current and potentiating compounds showing current $>150\%$ of control were selected, giving a hit rate of 5%.

Using the approaches detailed above, this assay has provided a robust screening platform for large compound collections and could easily be configured for screening other channels where leak subtraction cannot be applied.

3469-Pos Board B516

Highly Parallel Automated Patch Clamp Platform For High Quality Recordings From Up To 96 Cells At A Time

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Ion channels have for several years now, received more and more interest as drug targets, because of their known involvement in chronic and acute disease. The frequently cited fact that 15% of the 100 top selling drugs target ion channels, motivates the pharmaceutical industry to search for techniques that accurately can determine ion channel function, associated with an acceptable data throughput and reasonable cost per data point. Patch clamp is the gold standard for obtaining highly relevant information about ion channels and their effectors, but is an extremely laborious technique. This has spurred the development of automated patch clamps that are capable of high quality recordings, at a much higher throughput. Up to now, several automated patch clamp platforms are commercially available which allow for high quality recordings. None of these meet the throughput requirements given by high throughput screening in drug discovery.

To meet the ever increasing demand for higher throughput in ion channel screening and safety testing we have developed a highly parallel patch clamp platform. The platform supports giga-seal recordings, continuous recording during compound application and addition of multiple compounds at each of the 96 cells recorded from at a time. The platform allows recordings in the whole-cell configuration with high success rates. Due to the open structure of the patch clamp substrate, dose-responses can be obtained, thus cutting the costs per data point to be compatible to industrial requirements. Data from different cell types and ion channels will be presented.

3470-Pos Board B517

Action Potential Peak Shape Analysis As A New Tool For Antiarrhythmic Drug Development

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Cardiac arrhythmia is a critical heart condition characterized by abnormal electrical activity of the heart. There are a wide variety of drugs which are approved for the treatment of arrhythmia; many of them are acting on voltage dependent sodium, potassium or calcium channels. One of the problems concerning determination of the major mechanism of action of antiarrhythmics is that measurement of their effects on the different ion channels is time-consuming and usually done utilizing different experimental conditions. In order to counter this problem, in this study we applied a novel method, action potential shape analysis, to determine the effect of selected antiarrhythmics on voltage dependent sodium, potassium and calcium channels without performing time-consuming voltage-clamp experiments on each ion channel. Our method is based on fitting ion channel parameters to intracellularly or extracellularly recorded action potentials in a realistic model of NG108-15 cells and quantifying drug effects through their action on the shape of the action potentials and consequently on the fitted ion channel parameters. For this study we selected four drugs, quinidine, lidocaine, encanide, and amidarone, representing Class Ia, Ib, Ic and Class III antiarrhythmics, respectively. Quinidine, encanide and amidarone blocked both sodium and potassium channels, while lidocaine, at the measured membrane potential, shifted the activation of sodium channels in a depolarizing direction. Amidarone showed profound calcium channel blocking properties. Our work is a first step towards establishing a new assay system, based on the analysis of the shape of intracellularly and eventually extracellularly recorded action potentials, which can be used for fast quantitative analysis of drug effects on ion channel currents and classification of antiarrhythmics, and also for measurement of possible cardiac side effects of drug candidates

3471-Pos Board B518

MarkoLAB: A Graphical Interface To Study Stochastic Channel Behavior

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The most studied feature of an ionic channel is the current flowing through it. This happens when the channel moves into the open state, but most of the time, as revealed by the low open probability values, the channel transitions between closed or inactive states. By focusing only when the channel enters or leaves the open state the most of its activity is being missed. To have a better representation of the total behavior of the channel we constructed a computer program (MarkoLAB) that creates a 3-D plot with all channel's states. In this graphical interface each state is represented with a column. The height of the column is proportional to the occupancy level. During voltage clamp simulation the transition between states are visualized as changes in the columns' height. This dynamic plot provides more complete information about the channel behavior and illustrates the stochastic nature of the transitions. Furthermore the macroscopic current is simultaneously shown allowing the user to link the single channel activity with the overall result of the ensemble currents. The program was developed in LabVIEW language and the stochastic transitions were implemented with a Monte Carlo simulation. This first version of the software covers three typical channels under voltage clamp conditions: The rapid sodium channel (I_{Na}), the slow activated potassium channel (I_{Ks}) and the L-type calcium channel. (I_{CaL}). MarkoLAB is an original tool to gain insight of the channel kinetics and illustrates more clearly concepts as recovery from inactivation or distinguish between voltage-dependent versus calcium-dependent inactivation. This novel representation of channel activity constitutes a powerful aid to demonstrate effect of gene mutations or drugs on the channel function.