of antibiotics through porins (OmpF, OmpC) was done to elucidate the uptake kinetics of antibiotics through porins.
One main set of experimental data to be presented is on connexins proteins. Connexins are widely distributed in mammalian tissues and serve to join cells together into larger, functional units. We investigated the properties of hemichannels from Cx26 and Cx43 which were isolated biochemically and reconstituted into synthetic lipid membranes. In this study, preliminary data suggest the formation of gap junctions between cells and synthetic bilayer membranes. This opens possibilities to access the cytoplasm of living cells for biochemical or electrical studies, and especially to develop novel automated techniques for electrophysiological studies.

3688-Pos
Viral and Host Channels: A Comparison
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Viruses and their host cells have something in common they both need and encode ion channels. Whilst for the host the role and the mechanism of function of these membrane proteins is straight forward, knowledge about the viral channels seems just to unroll on the molecular level. Experimental tools are gradually delivering low and high resolution structures with computational methods as another source for structural information on the atomic level.
The viral membrane proteins identified as channels are becoming increasingly more complex in respect to their topology. Most of the channels are still very much smaller than the channels of the host. This triggers an intrigued discussion about (i) when is a membrane protein a channel and (ii) what do the smaller viral channels have in common with their bigger class mates, the host channels. Data from computational modeling will be presented along these lines.

3689-Pos
Channelrhodopsin-2 Variants with Accelerated and Decelerated Channel Kinetics
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The light-activated cation channel Channelrhodopsin-2 (ChR2) is a powerful tool for controlling neuronal activity. Its genetic information is carried into neurons which express the protein. Channel activation by blue light exposure causes membrane depolarizations that immediately trigger action potentials. We genetically modified ChR2 wildtype and created variants with decelerated and accelerated channel kinetics as well as changed ion selectivities. ChR2 mutations with new features broaden the toolbox for neuroscientists but the mechanisms of channel activation and ion translocation are still unclear. We use a combination of theoretical approaches like molecular and mathematical modeling as well as experimental techniques like UV/vis spectroscopy, flashlight photolysis and two electrode voltage clamp to reveal how mutations affect the channel properties.

3690-Pos
VSOP/Hv1 Proton Channels Sustain Superoxide Production, Calcium Entry, and Cell Migration by Limiting the Depolarization and Acidification of Activated Neutrophils
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Neutrophils kill microbes with superoxide radicals generated by the NADPH oxidase, an enzyme that moves electrons across membranes. Voltage-gated proton channels (VSOP/Hv1) are required for high-level superoxide production by phagocytes, but the mechanism of this effect is not clear. Using mice bearing a targeted disruption in the VSOP/Hv1 gene (VSOP/Hv1-/-), we show that neutrophils devoid of VSOP/Hv1 lack proton currents but have normal electron currents, indicating that these cells have a fully functional oxidase that cannot conduct protons. VSOP/Hv1-/- neutrophils were more acidic and more depolarized than neutrophils from wild-type mice, and consequently produced less superoxide. Loss of VSOP/Hv1 also aborted calcium responses to chemoattractants, increased neutrophil spreading, and decreased chemokinesis. Our findings indicate that proton channels extrude the acid and compensate the charge generated by the oxidase, thereby sustaining calcium entry signals that control the adhesion and motility of neutrophils. Loss of proton channels thus aborts superoxide production and causes a severe signalling defect in neutrophils.

3691-Pos
Design of a Potent and Selective Small Molecule Kv1.5 Blocker
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The voltage-gated potassium channel Kv1.5 is being studied extensively as a potential target for treating atrial fibrillation and other life-threatening arrhythmias. Since Kv1.5 is expressed selectively in the human atrium and not in the ventricle, a potent and selective Kv1.5 blocker should therefore significantly increase the action potential duration (APD) of the atrium without affecting that of the ventricle. Unlike the existing anti-arrhythmic drugs such as amiodarone, sotalol etc. that block the Kv11.1 channel (hERG), a potent and selective Kv1.5 blocker should not induce dangerous and fatal proventricular arrhythmia.
Phenoxyalkoxypsoralens (PAPs) are a class of compounds that has previously been described to block both the lymphocyte Kv1.3 and the cardiac Kv1.5 channel (*Mol. Pharmacol. 2005*). Through a combination of classical medicinal chemistry and traditional electrophysiology, we now studied the structure-activity relationship of PAPs with the aim of generating more selective Kv1.5 blockers. When the side chain phenyl ring of PAPs were decorated with a combination of electron-donating (methyl) and electron-withdrawing (nitro) groups as in PAP-22 {5-[3-(4-methyl-2-nitrophenoxy)-propoxy]psoralen}, the compounds exhibited a four to five fold increase in selectivity for Kv1.5 over Kv1.3. However, when we substituted the nitro group with a chloro group, as in PAP-25 {5-[2-chloro-4-methylphenoxy)propoxy]-psoralen}, more selective Kv1.3 blockers were generated. We are currently further investigating the effect of other strongly electron-withdrawing groups instead of the nitro group in order to increase the potency and selectivity of PAPs for Kv1.5 over Kv1.3. Other fused tricyclic rings containing 2-aminobenzothiazole are also being explored as a potential pharmacophore to design and develop Kv1.5 blockers.
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3692-Pos
Using Domain Based Discovery Methods to Identify Prokaryotic Counterparts to Eukaryotic Protein Ion Channels
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Our lab, in collaboration with the laboratory of I. Aravind, used domain based methods to discover the prokaryotic counterparts of Ach receptor channels (Tasneem et al), after whole-protein approaches such as BLAST had failed. Our identification was verified by functional studies and by x-ray crystallization of targets we identified. We have subsequently streamlined and formalized the domain search methods and applied them to a variety of ion channels and other membrane proteins. It has become clear that domain based methods are more powerful than whole-protein approaches, provided one has good domain definitions to start the search. The use of domain-based methods depends on a somewhat different model of evolution from BLAST. In BLAST, the operational model is the substitution of one amino acid for another, with gaps being treated as a particular type of substitution. Domain-based methods deal readily and directly with the phenomenon of large scale reorganization of domains, which is now recognized as an essential process for innovation in evolution. In this presentation we will provide an update on prokaryotic Ach receptor channels, a survey of prokaryotic glutamate receptor channels and their relationship to their eukaryotic counterparts, the discovery of a prokaryotic counterpart to HCN and CNG channels which appears likely to be closely related to their common ancestor, and searches on other families that are under way at the time of preparation of this abstract. We gratefully acknowledge support from NSF grants 0835718 and 0235792, from NIH grants 5PN2EY016570-06 and 5R01NS063405-02 from the Beckman Institute for Advanced Science and Technology, the National Center for Supercomputing Applications, and the Renaissance Computing Institute.

3693-Pos
A Novel Fluorescence Assay for Voltage-Gated Ion Channels Based upon Light Induced Voltage Clamp
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Ion channels are a key target class with a high therapeutic potential in virtually all possible disease indications. In addition, a potential side effect of pharmaceutical compounds is the blocking of hERG channels in heart cells making easy and cost effective hERG safety screening necessary for drug development today. Conventional screening techniques yield insufficient data quality particularly when assessing voltage-gated ion channels. Thus, the development of new reliable technologies is desirable to integrate ion channel screening into early lead generation stages of drug discovery.

Here we demonstrate a method that allows light-induced activation of voltage-gated ion channels and the concurrent imaging of membrane potential changes using voltage-sensitive dyes. This light-induced voltage clamp (LIVC) method uses photostimulation through channelrhodopsin-2 (ChR2) to activate voltage-gated ion channels. ChR2 allows light to be immediately transduced into a depolarizing ionic current, which in turn causes voltage-gated ion channels to open. In our system we coexpressed ChR2 either with the voltage-gated potassium channels hERG or hKv1.5 in cell lines and in Xenopus oocytes. In electrophysiological experiments we show that light-induced depolarization through ChR2 sufficed to activate hERG as well as hKv1.5 channels. We were further able to optically monitor the light-induced membrane de- and hyperpolarizations on a millisecond timescale with the voltage-sensitive RH421 and Annine6. The fluorescence readout reflected the dose-response relationships of the hERG blocker Terfenadine and the hKv1.5 inhibitor DPO-1 obtained from patch-clamp measurements.
LIVC represents a solely optical technology with remote activation of the target voltage-gated ion channels by the delivery of a flash of blue light and simultaneous detection of their activity employing voltage-sensitive dyes. It combines the high-throughput of optical methods with the high-content of patch clamp concerning high temporal resolution, membrane potential control and repetitive stimulation.

3694-Pos

Functional Studies of Volvox Channelrhodopsin Chimeras

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Recently, two new members of the channelrhodopsin family have been discovered in the multicellular green alga Volvox carteri and named VChR1 and VChR2. VChR2 shows a similar characteristic to the known Chlamydomonas ChR2 [1], which is successfully employed in optogenetics. It shows a maximum absorption at 470nm and can be well expressed in eukaryotic cells like HEK293T cells. In contrast, VChR1 shows the most red-shifted absorption peaking at 540nm. It has been successfully used to depolarize hippocampus cells even at 590nm [2].
Longer wavelengths would enable deeper brain stimulations and would therefore be less invasive and harmful for tissues. The use of VChR1 was hindred by low expression levels and membrane localization. In this study, we systematically exchanged helices, creating chimeras of VChR1 and VChR2. Functional chimeras show differences in expression levels, membrane localization and absorption. Several chimeras show higher expression levels and plasma-membrane localization in HEK293 cells than VChR1 while preserving absorption at 540nm.
Thereby we were able to identify key determinants causing the colour-shift from VChR1 to VChR2, located in the last three helices of these proteins. Mutagenesis of the relevant amino acids that are thought to be promising candidates for fine-tuning to longer wavelength absorption.
[1] Channelrhodopsins of Volvox carteri are photochromic proteins that are specifically expressed in somatic cells under control of light, temperature, and the sex inducer. Kianianmomeni A, Stehfest K, Nematollahi G, Hegemann P, Hallmann A. Plant Physiol. 2009 Sep;151(1):347-66.
[2] Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. Zhang F, Prigge M, Beyrière F, Tsunoda SP, Mattis J, Yizhar O, Hegemann P, Deisseroth K. Nat Neurosci. 2008 Jun;11(6):631-3.

3695-Pos

K2P1 Assembles with K2P3 or K2P9 to Form Sumo-Regulated Task Background Channels

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TASK subunits K2P3 and K2P9 form homo- and hetero-dimeric channels in neurons with varied sensitivities to anesthetics, flavors and pH (Berg et al. J Neurosci. 2004. 24:6693-702; Bautista et al. Nat. Neurosci. 2008. 11:772-9). Here, K2P1 subunits (Rajan et al. 2005. Cell 121, 37-47) are shown to confer SUMO-regulation on TASK channels in rat cerebellar granule neurons (CGN). First, CFP (C) or YFP (Y)-tagged subunits studied in CHO cells by donor-decay Forster resonance energy transfer (FRET) confirm biochemical evidence for assembly of K2P2 and its native isoform (Thomas et al., Neuron 2008 58:859-70). Next, FRET shows association of C-K2P1 and Y-K2P1, Y-K2P3 or Y-K2P9 but not Y-K2P2 or Kv2.1. As expected from Rajan et al, FRET registers association of Y-SUMO and C-K2P1 but not C-K2P3 or C-K2P9. In contrast, Y-SUMO and C-K2P3 or C-K2P9 FRET when untagged K2P1 is co-expressed. Consistent with electrophysiological studies (EP) showing one SUMO per channel is sufficient to silence K2P channels (Plant et al. this meeting), channels with two linked K2P1 subunits (WT-WT), WT and SUMO-insensitive K2P1 (WT-K274Q), WT and K2P3 (WT-K2P3) or WT and K2P9 (WT-K2P9), pass currents when membrane patches are exposed to SUMO-protease (SENP1) and silenced by SUMO1. In contrast, K2P3-K2P3, K2P9-K2P9, K2P3-K274Q or K2P9-K274Q are constitutively active and insensitive to SENP1 and SUMO1. Finally, in CGN, immunochemistry shows K2P1, K2P3, K2P9, SUMO, SUMO E1 conjugase and SUMO E2 ligase in plasma membrane; EP reveals IK_{SO} regulation by SENP1 and SUMO, and transfection with mutant subunits demonstrate assembly of K2P1 with K2P3 or K2P9 by FRET and EP.

Excitation-Contraction Coupling II

3696-Pos

The Effect of Apelin on Single Isolated Cardiac Myocytes from Wild-Type and Apelin / APJ KO Mice

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Apelin, an endogenously produced peptide discovered in 1998, is regarded as one of the most potent substances to increase net contractile output in isolated heart and whole animals, apparently in a load-dependent manner. Unlike other inotropic agents, apelin has not been shown to cause cardiac hypertrophy. However, little is known about its cellular effects, with only two papers on isolated cardiomyocytes, neither of which imposed external mechanical constraints. This study assesses dynamic parameters of cardiomyocytes from wild type (WT), apelin-receptor knockout (AJP-KO) and apelin knockout (Ap-KO) mice. In addition to steady-state parameters such as fractional shortening, we measured time to peak contraction (TTP), and maximum velocities of contraction and relaxation.
Apelin administration to unloaded cells did not change fractional shortening, but significantly ($p<0.05$) decreased TTP both in WT ($93.1\% \pm 2\%$, n=90) and Ap-KO cardiomyocytes ($83.1\% \pm 5\%$, n=14). The specificity of apelin effects was verified in AJP-KO myocytes, where no significant change in TTP was seen ($98.2\% \pm 2\%$, n=69).
After application of mechanical pre-loads, using the two-carbonfibre technique, TTP reduction was enhanced in Ap-KO myocytes ($64.6\% \pm 5\%$, n=11). To characterise apelin effects on developed tension, the ratio between the slopes of end-systolic and end-diastolic tension-length relations was obtained. This is a non-dimensional parameter, termed Frank-Starling-Gain (FSG), that is independent of cell cross-section and allows inter-individual comparisons. Under control conditions, FSG was 1.36 ± 0.05 (n=10). After application of 10nM apelin this rose to 1.96 ± 0.23 (n=6, $p<0.05$), indicating improved net contractile output. Interestingly, this was caused largely by a slope reduction of the end-diastolic tension-length relation. This could explain the combination of increased net force output with lack of hypertrophy induction, and suggests that apelin more appropriately be described as a positive lusitropic agent, rather than an inotropic one.

3697-Pos

Mechanism of the Spontaneous Beating of Skeletal-Based Precursor of Cardiomyocytes (SPOC)

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The skeletal muscle-based precursor of cardiomyocytes (SPOC), a stem cell that potentially differentiates into heart cells [Winitsky et al. (2005) PloS Biol 3(4), e87], were isolated from leg skeletal muscle of the rat. Initially the SPOC cells had round and small appearance. When cultured on the dishes coated with extracellular matrix, they differentiated into round, tear drop, or tubular shaped cells. During the course of their differentiation, the SPOC cells exhibited a rhythmic beating associated with spontaneous oscillation of the intracellular Ca^{2+}. The spontaneous beating could be suppressed by Cd^{2+} and nifedipine, indicating possible contribution of the L-type Ca^{2+}-channel to the development of the automaticity. The rate of the spontaneous beating was accelerated by the administration of isoproterenol. Immunocytochemistry of the SPOC cells indicated the existence of Nkx 2.5, cardiac TnT, dihydropyridine receptor, and ryanodine receptor. Action potentials during the spontaneous beating and the underlying membrane currents were analyzed by using the patch-clamp technique. Our results indicate that the SPOC cells develop their automaticity by a unique mechanism distinct from that of skeletal or heart muscle cells.