indeed fixed by counting subunits in many individual complexes using TIRF microscopy (Ulbrich and Isacoff, 2007, 2008). We expressed GFP-tagged KCNQ1 or KCNE1 in *Xenopus* oocytes at low density and counted bleaching steps in many fluorescent spots corresponding to single channel complexes. First, we confirmed that KCNQ1 forms a tetramer. Next we counted GFP-tagged KCNE1 subunits co-expressed with mCherry-tagged KCNQ1. We observed up to four bleaching steps from GFP-KCNE1 co-localized with mCherry, indicating that up to four KCNE1 subunits can bind to one KCNQ1 tetrameric channel. We find that the number of KCNE1 subunits per complex increases as the expression of KCNE1 is raised relative to that of KCNQ1. Our results suggest that modulation of KCNQ channels may be regulated by the level of expression of KCNE subunits.

708-Pos

Structural Underpinnings for Modulation of the Voltage-Gated Potassium Channel KCNQ1 by the KCNE Family of Proteins

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The voltage-gated potassium channel KCNQ1 is modulated by KCNE1 to form the I_{Ks} current involved in cardiac repolarization. KCNE1 is the best characterized member of a family of modulatory proteins which impart distinct ion channel physiology. Mutations in KCNQ1 and familial KCNE proteins have been linked to human diseases including congenital deafness and congenital long QT syndrome, which is an inherited predisposition to potentially life-threatening cardiac arrhythmias. The biophysical basis of the KCNE1 modulation of KCNQ1 has been previously characterized in our lab with an interdisciplinary approach utilizing nuclear magnetic resonance (NMR) spectroscopy, electrophysiology, biochemistry, and computational biology. In this work we extend the characterization to include two other family members; namely, KCNE3 and KCNE4. KCNQ1 homology models and the KCNE family proteins KCNE1, KCNE3, and KCNE4 are used as the basis of a comparative study to deduce the molecular mechanisms of voltage-gated potassium channel regulation by these accessory subunits. KCNE1 binds to KCNQ1 and causes delayed channel activation and increased conductance, while, KCNE3 promotes rapid and increased conductance in KCNQ1. On the other hand, KCNE4 binding causes a strict inhibition of KCNQ1 conductance. In this work we present data that suggests the structural biological basis for how the homologous KCNE1, KCNE3, and KCNE4 proteins modulate KCNQ1 in such starkly contrasting manners. *This work was supported by NIH grant R01DC007416.*

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KCNE4 Juxtamembrane Region Interacts with Calmodulin and is Necessary for KCNQ1 Modulation

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Voltage-gated potassium (K_V) channels are modulated by the KCNE family of single transmembrane proteins. A membrane-based yeast two-hybrid screen to discover KCNE4 interacting proteins identified calmodulin (CaM) as a candidate. Previous studies demonstrated that CaM binding to KCNQ1 is required for functional expression of KCNQ1-KCNE1 channels *in vitro*, and increasing concentrations of intracellular calcium stimulate KCNQ1-KCNE1 channels in *Xenopus* oocytes in the presence of wild-type CaM but not mutant CaM that cannot bind calcium. We have tested the functional consequences of the interaction between KCNE4 and CaM with the hypothesis that KCNE4 modulation of K_V currents may depend on its interaction with CaM. We validated the biochemical interaction between KCNE4 and CaM using CaM-agarose pull-down, and tested KCNE4 mutants that targeted putative CaM binding sites. Mutation of a juxtamembrane site (L[69-72]A) exhibited near complete disruption of CaM binding, whereas biotinylation studies performed in CHO cells confirmed expression of the mutant protein at the cell surface. The ability of L[69-72]A to modulate KCNQ1 was then studied using whole-cell patch clamp recording to determine if functional consequences accompany the loss of CaM binding. Wild-type KCNE4 completely inhibits potassium current in CHO cells transiently co-transfected with KCNQ1, but cells co-expressing KCNQ1 with L[69-72]A exhibited KCNQ1-like currents. Mean ($\pm$ SEM) current density (measured during step to +60 mV from holding potential of −80 mV) in cells expressing KCNQ1 alone was 37.0 $\pm$ 4.25, not significantly different from cells co-expressing KCNQ1 with L[69-72]A (32.1 $\pm$ 3.3), but significantly different from cells co-expressing KCNQ1 with wild-type KCNE4 (3.3 $\pm$ 0.35). These studies suggest that a juxtamembrane region in KCNE4 is critical for its interaction with CaM and is necessary for modulation of KCNQ1.

710-Pos

Molecular Mechanisms Underlying Membrane Potential-Mediated Regulation of Neuronal K_{2P}2.1 Channels

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The activity of background K_{2P} channels adjusts the resting membrane potential to enable plasticity of excitable cells. Here we have studied the regulation of neuronal human K_{2P}2.1 (KCNK2, TREK-1) channel activity by resting membrane potential. When heterologously expressed in *Xenopus laevis* oocytes, K_{2P}2.1 currents gradually increased several fold at hyperpolarizing potentials and declined several fold at depolarizing potentials, with a midpoint potential of −60 mV. As K_{2P} channels are not equipped with an integral voltage sensor, we sought extrinsic cellular components that could convert changes in the membrane electrical field to cellular activity that would indirectly modify K_{2P}2.1 currents. K_{2P}2.1 voltage sensitivity was found not to be mediated by the activity of either voltage activated calcium channels, the *Xenopus* voltage sensitive proton channel (Xl-Hv) or the *Xenopus* voltage sensor-containing phosphatase (Xl-VSP). On the other hand, we report that membrane depolarization activated the Gq protein-coupled receptor pathway, in the apparent absence of ligand, resulting in phosphatidylinositol-4,5-bisphosphate (PIP_2) depletion through the action of phospholipase C. Our results suggest a novel mechanism in which an indirect pathway confers membrane potential regulation onto channels that are not intrinsically voltage-sensitive to enhance regulation of neuronal excitability levels. The ability of these proteins to operate without any external ligand enhances plasticity at the single cell level, independent of higher regulatory pathways at the tissue or even the organism levels.

711-Pos

K^+ Channel Interacting Proteins 2, 3 and 4 are Critical Components of Kv4 Channel Complexes in Cortical Pyramidal Neurons

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The rapidly activating and inactivating voltage-gated K^+ (Kv) current, I_A, is critical for many neuronal functions, including repetitive firing and synaptic integration. Previous studies revealed that in cortical pyramidal neurons the majority of I_A is encoded by Kv4.2 and Kv4.3 α-subunits. Little, however, is known about the functional roles of K^+ Channel Interacting Proteins (KChIP) 1, 2, 3, and 4 in the generation of I_A. Biochemical experiments revealed that KChIPs 2, 3 and 4 (2-4) co-immunoprecipitate with Kv4.2 in samples from mouse cortex suggesting roles for these three KChIPs in the generation of functional Kv4-encoded channels in cortical pyramidal neurons. Electrophysiological experiments conducted on cortical pyramidal neurons from mice ($KChIP3^{-/-}$) harboring a targeted disruption of the KChIP3 locus revealed that I_A densities and properties were similar to wild type neurons. Interestingly, in cortical samples from $KChIP3^{-/-}$ mice the protein levels of KChIP 2 and 4 were increased suggesting functional compensation for the loss of KChIP3. Similarly, in $KChIP2^{-/-}$ cortices KChIP3 and 4 protein levels were increased relative to wild type. Concurrently knocking down the expression of KChIPs 2-4 using RNAi constructs targeting each of the three KChIPs induced a reduction in I_A density consistent with roles for KChIPs 2-4 in the generation of native Kv4-encoded I_A channels. In cortical samples from $Kv4.2^{-/-}$ and $Kv4.3^{-/-}$ mice, the protein expression levels of KChIPs 2-4 were decreased. Additionally, in samples from mice lacking both Kv4.2 and Kv4.3 KChIP2-4 proteins were barely detectable. Taken together these results demonstrate that KChIPs 2-4 associate with Kv4.2 and Kv4.3 in cortical neurons, this association stabilizes KChIP proteins and, in addition, that KChIPs 2-4 are critical components of native Kv4 channels in cortical pyramidal neurons.

712-Pos

HMR 1098 is not an Sur Isotype Specific Inhibitor of Sarcolemmal or Heterologous K_{ATP} Channels

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Murine ventricular and atrial K_{ATP} channels contain different sulfonylurea receptors (ventricular K_{ATP} channels are Kir6.2/SUR2A complexes, while atrial K_{ATP} channels are Kir6.2/SUR1 complex). HMR 1098, the sodium salt of HMR 1883 {1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea}, has been used as a selective sarcolemmal (i.e. SUR2A-dependent) K_{ATP} channel inhibitor. However, specificity for ventricular versus atrial channels has not been examined. We used whole-cell patch-clamp techniques on mouse ventricular and atrial myocytes as well as $^{86}Rb^+$ efflux assays and excised inside-out patch-clamp techniques on Kir6.2/SUR2A and Kir6.2/SUR1 channels heterologously expressed in COSm6 cells. In mouse ventricular myocytes, pinacidil-activated K_{ATP} currents were inhibited

by HMR 1098 at high (100 ∈μM) but not low (10 ∈μM) concentration. By contrast, in atrial myocytes, both spontaneously activated and diazoxide-activated K_{ATP} currents were effectively inhibited by 10 ∈μM HMR 1098. Consistent with this finding, HMR 1098 inhibits $^{86}Rb^{+}$ effluxes through Kir6.2/SUR1 more effectively than Kir6.2/SUR2A channels in COSm6 cells. In excised inside-out patches, HMR 1098 effectively inhibited Kir6.2/SUR1 channels as well as Kir6.2/SUR2A channels in the absence of nucleotides, but inhibited Kir6.2/SUR1 channels more effectively than Kir6.2/SUR2A channels in the presence of MgADP and MgATP (mimicking physiological stimulation). Finally, dose-dependent enhancement of insulin secretion from pancreatic islets confirms that HMR 1098 is an effective inhibitor of Kir6.2/SUR1-composed K_{ATP} channels, and is not specific for SUR2A-composed channels.

713-Pos

Epac-Mediated Mobilization of Intracellular Calcium in Vascular Myocytes and the Downstream Effects on Arterial K_{ATP} Channels

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Exchange proteins directly activated by cyclic AMP (Epac or cAMP-GEFs) are a family of novel cAMP-binding effector proteins [1]. Using the Epac-specific cAMP analogue 8-pCPT-2-*O*-Me-cAMP we show cAMP-mediated but PKA-independent mobilization of $Ca^{2+}{}_i$ within vascular myocytes and downstream effects that culminate in the inhibition of ATP-sensitive potassium (K_{ATP}) channels.

Application of 8-pCPT-2-*O*-Me-cAMP (5μM) caused a 41.6 ± 4.7% inhibition of pinacidil-evoked whole-cell K_{ATP} currents recorded in isolated rat aortic smooth muscle cells. Inclusion of the Ca^{2+} chelator BAPTA (20μM) in the pipette-filling solution reduced the inhibition to 8.7 ± 4.4%, consistent with the idea that Epac mediates its effects by elevating $[Ca^{2+}]_i$. In support of this, 8-pCPT-2-*O*-Me-cAMP (5μM) caused a transient 171.0 ± 18.0nM increase in $[Ca^{2+}]_i$ in Fura-2-loaded myocytes, which persisted in the absence of extracellular Ca^{2+}. Caffeine-induced Ca^{2+} transients triggered in the presence of 8-pCPT-2-*O*-Me-cAMP typically showed a secondary Ca^{2+} increase, reminiscent of ectopic Ca^{2+} transients observed in Epac-activated cardiac myocytes [2]. While Ca^{2+} transients returned to baseline after 15-20s, the inhibition of K_{ATP} current was sustained, suggesting that Ca^{2+}*per se* does not affect channel activity and implicating the involvement of Ca^{2+}-activated enzymes. Preincubation with calcineurin inhibitors cyclosporin A (10μM) and ascomycin (5μM), significantly reduced the ability of 8-pCPT-2-*O*-Me-cAMP to inhibit K_{ATP} currents (inhibition 10.8 ± 2.8% and 7.3 ± 1.6%).

These findings suggest cAMP-mediated Epac activation in vascular smooth muscle mobilizes Ca^{2+} from internal stores and inhibits K_{ATP} channels through the activation of the Ca^{2+}-sensitive enzyme, calcineurin.

1. Bos JL (2006). *Trends in Biological Sciences***31**:680-686
2. Hothi et al (2008). *Pflugers Archiv.***457**:253-270.

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Neuronal Systems & Modeling

714-Pos

Membrane Potential Imaging in Neurons using Fluorinated Voltage-Sensitive Dyes and a Custom Multiphoton Brain Slice Microscope

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In order to fully understand the physiology of fundamental neurophysiological processes such as synaptic integration and synaptic plasticity, direct recording of changes in membrane potential neuronal dendrites and spines is essential. In an effort to improve voltage-sensitive dye measurements of synaptic potentials and backpropagating action potentials, our group has developed new fluorinated dyes with enhanced photostability. We have also made performance improvements on our custom, non-linear optical microscope for greater sensitivity. By modifying a commercial Zeiss microscope we have added two ''up front'' epifluorescence detection channels and one transfluorescence detection channel. Optics for these new light paths were optimized using numerical ray tracing. Here we show that we are able to fill individual neurons with these dyes via somatic patch pipettes and record membrane potential changes in the soma and dendrites of Purkinje neurons in cerebellar brain slices. Using voltage clamp protocols, membrane potential was changed in a stepwise fashion, resulting in changes in membrane fluorescence. When excited with 1060 nm light, the new dyes typically produced changes in fluorescence (dF/F) between 3 and 7 % for 50 mV changes in membrane potential. Feasibility of using second harmonic generation to record membrane potential with these dyes was investigated in a cultured cell line by measuring dSHG/SHG, kinetics, and intensity as a function of dye concentration.

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715-Pos

Two-Photon Optogenetic Control of Neuronal Activity with Single Synapse Precision by Sculpted Light

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Recent advances in optogenetic techniques have provided new tools for controlling neuronal activity, opening up the way to a range of studies in neuroscience. The most widely used approach has been the optical activation of the genetically expressed light-gated ion channel Channelrhodopsin-2 (ChR2) to initiate population activity in neuronal circuits. However, single cell resolution of optogenetic activation has remained challenging. This is because neither single-photon nor conventional two-photon excitation provides the necessary combination of high spatial selectivity and the simultaneous stimulation of a sufficiently large membrane area necessary to induce fast and significant depolarizations by ChR2 in a single neuron.

The presented work reports on two-photon excitation of ChR2 allowing the generation of fast and large ChR2-mediated currents in single cells with high spatial and temporal resolution by using temporally focused beams. It is demonstrated that this technique efficiently induces strong depolarization and reliable action potential firing in single ChR2-expressing neurons in rat and mouse hippocampal slices. It is further shown that subcellular compartments such as dendrites and large presynaptic terminals can be activated by the TF-2P technique. The superb spatial and temporal resolution provided by this technique allows so far unattainable precision for fine manipulation of neuronal activity to study and control the function of neuronal microcircuits *in vitro* and *in vivo.*

716-Pos

Combining Optical Tweezers, Laser Microdissectors and Multichannel Electrophysiology for the Non-Invasive Tracing and Manipulation of Neural Activity on Single Cell and Network Level

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During differentiation, cell processes initiate exploratory motion to create connections with other cells thereby creating a tissue architecture that is capable of performing complex tasks. The interplay between mechanical and chemical stimuli seems necessary for triggering the proper biochemical reactions that eventually lead to the functional organization of cells and tissue[1].

There are different approaches for studying the focused mechanical-chemical transduction, either at single cell[2] level or at tissue[3] level. To better understand tissue development (cell differentiation, cells contact formation, tissue organization), we would like to bridge the gap between experiments on single cells and complex tissues. Therefore we are developing a system for combining optical techniques such as optical tweezers[4] and a laser dissector with electrophysiological tools. Optical tweezers permit to apply localized mechanochemical stimuli onto cells[5] while a laser dissector can alter individual neuronal connections[6]. By adopting neuronal networks as a biological model, neural signal transmission affected by such external stimuli can be recorded non-invasively by microelectrode arrays.

Ongoing work is targeted at correlating the temporary or lasting changes in neural networks to the type and site of the stimulus.

1. Pampaloni, F. et al. Nat. Rev. Mol. Cell Biol. 8, (2007).
2. Peyton, S.R. et al. Cell Biochemistry and Biophysics 47, (2007).
3. Judex, S. et al. J. Biomech. 30, (1997).
4. Neuman, K.C. & Block,S.M. Review of Scientific Instruments 75, (2004).
5. Cojoc, D. et al. PLoS. ONE. 2, (2007).
6. Colombelli J. et al. Methods Cell Biol. 82, 267(2007).

717-Pos

Astrocytic Processes Ensheathing Synaptic Glomeruli cause Anomalous Extracellular Diffusion

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Brain extracellular space forms a channel where signaling molecules, growth factors and therapeutics diffuse. Characterization and quantification of the extracellular diffusion is thus important for intercellular signaling and drug delivery. We have recently reported that the extracellular diffusion is *anomalous* in the granular layer of rat cerebellar slices (anomalous diffusion model quantified an average anomalous exponent $d_w = 5.0$). In this respect, the granular layer significantly differs from most brain regions where the extracellular diffusion is *normal* (i.e., $d_w \approx 2.0$). In biological systems, anomalous diffusion may