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

Gregor I. Purves, Tomoko Kamishima, Lowri M. Davies, John M. Quayle, Caroline Dart.
University of Liverpool, Liverpool, United Kingdom.

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.

Supported by the BHF

Neuronal Systems & Modeling

714-Pos

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

Corey D. Acker, Leslie M. Loew.
Richard D. Berlin Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, CT, USA.

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.

Work funded by the following NIH grants: R01-EB001963 and P41-RR013186.

715-Pos

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

Alipasha Vaziri.
Howard Hughes Medical Institue, Ashburn, VA, USA.

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

Francesco Difato, Emanuele Marconi, Alessandro Maccione,
Luca Berdondini, Evelina Chieregatti, Giuseppe Ronzitti,
Fabio Benfenati, Axel Blau.
Italian institute technology, genova, Italy.

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

Fanrong Xiao, Sabina Hrabetova.
SUNY Downstate Medical Center, Brooklyn, NY, USA.

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