small junctional sarcoplasmic reticulum (SR). With improved resolution and sensitivity that can be achieved by examining Ca^{2+} spark - blink pairs, we report here for the first time small, local but also sometimes spatially extensive Ca^{2+} releases (subsparks and Ca^{2+} "mist") that co-exist with regular Ca^{2+} sparks. Similar low-level Ca^{2+} releases also occur in the declining phase of regular Ca^{2+} sparks and the abundance of these small Ca^{2+} releases dictates the kinetics of the spark-blink pair. We propose a model in which the Ca^{2+} release unit, consisting of a large array of type 2 ryanodine receptor (RyR2) Ca^{2+} release channels, underlies the initial high-flux release of a Ca^{2+} sparks. In contrast, rogue unconstrained RyR2s, which may display higher Ca^{2+} sensitivity but smaller Ca^{2+} flux, produce the Ca^{2+} quark-like or "quarky" local releases. The existence of the additional release mechanism provides new fundamental mechanistic understanding of cardiac Ca^{2+} signaling in health and disease.

109-Plat

Concerted Phosphodiesterase (PDE) Subtype Activity Modulates Ca^{2+} Influx Through L-type Ca^{2+} Channels To Regulate Spontaneous Firing of Rabbit Sinoatrial Node Cells (SANC)

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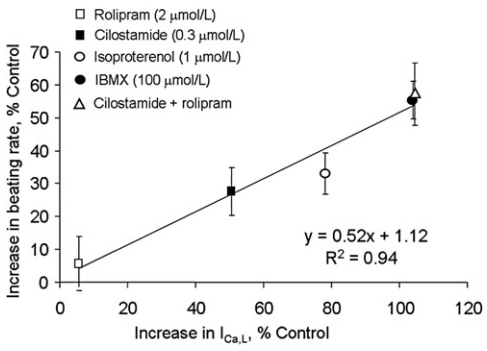
Spontaneous beating of rabbit SANC is controlled by cAMP-mediated, PKA-dependent rhythmic, local subsarcolemmal Ca^{2+}releases (LCRs) from sarcoplasmic reticulum during late diastolic depolarization. While Ca^{2+}influx via L-type Ca^{2+} channels ensures LCR occurrence, high basal PDE activity limits LCRs. The extent to which PDE regulates L-type Ca^{2+} current, $I_{Ca,L}$, however, remains enigma. We determined the extent of PDE subtype-dependent control of basal $I_{Ca,L}$, spontaneous SANC firing rate; and compared those to the effect of β-adrenergic receptor (β-AR) agonist, isoproterenol. A specific PDE4 inhibitor, rolipram, had no effect, on either $I_{Ca,L}$ or spontaneous beating; cilostamide, a specific PDE3 inhibitor, in contrast, increased both $I_{Ca,L}$ and spontaneous SANC firing (Fig). Simultaneous inhibition of PDE3 and PDE4 by (cilostamide+ rolipram) increased $I_{Ca,L}$; amplified LCR size (from 5.9 ± 0.58 to 8.6 ± 0.50 μm); decreased the LCR period (from 309.7 ± 20.6 to 214.3 ± 3.9 msec); and accelerated spontaneous SANC firing rate equivalent to broad-spectrum PDE inhibitor, IBMX. These effects were even greater than those produced by β-AR stimulation. Thus, concerted PDE3 and PDE4 activities control basal cAMP-PKA-dependent phosphorylation and suppress $I_{Ca,L}$, limiting basal LCRs and spontaneous SANC firing rate.



110-Plat

Development of Calcium Handling Defects During Aging in Spontaneously Hypertensive Rats

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Several defects in calcium handling have been identified in failing myocytes. We investigated the progression of these defects in the course of development of overt heart failure (HF). Intracellular Ca^{2+} transients were measured using confocal microscopy in intact hearts from age-matched Wistar-Kyoto (WKY) control rats and Spontaneously Hypertensive Rats (SHR) at 6, 7.5, 9 and >22 months of age. Amplitude of basal Ca^{2+} transients (cycle length of 700msec) in SHR increased at 7.5 and 9 months, but subsequently decreased at 22 months compared to WKY. SHR myocytes exhibited longer transient duration starting at 9 months compared to WKY. Cell-to-cell variability in transient duration increased at 7.5 months and subsequently decreased at 9 months as defects became more extensive. At 22 months, Ca^{2+} transients showed further increases in transient duration and intercellular variability. Restitution of Ca^{2+} release was slowed and was paralleled by increased Ca^{2+} alternans susceptibility starting at 9 months in SHR. Dyssynchronous alternans incidence increased beginning at 7.5 months in SHR compared to WKY. SHR myocytes also demonstrated an increased incidence of spontaneous Ca^{2+} waves at all ages, with the greatest difference at 22 months. A separate population of SHR myocytes showed Ca^{2+} waves that were activated during pacing (''triggered waves'') whose incidence increased at all ages but most profoundly at 22 months. We conclude that well-coupled failing myocytes demonstrate progressively increasing defects in Ca^{2+} cycling. Biphasic changes in calcium transient magnitude may partially account for a transient inotropic effect during compensation but ultimately reduced cardiac output in HF. The progression of Ca^{2+} handling defects may also account for the increasing sensitivity to alternans as well as incidence of spontaneous and triggered Ca^{2+} waves with age, possibly explaining increased arrhythmias during progressive HF.

111-Plat

Decreased Arrhythmia Probability After Exercise Training In Post Infarction Heart Failure

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Background: Approximately half of all deaths in heart failure patients are attributed to sudden death or ventricular fibrillation (VF). Exercise training might protect against arrhythmias and VF, but the mechanism is not explored. The aim of this study was to measure how exercise training modulates VF in post myocardial infarction heart failure (HF) and further explore the cellular mechanisms.
Methods: We compared HF rats subjected to either moderate or high intensity exercise training with HF sedentary and sham. VF threshold and action potential duration (APD) were measured in isolated hearts, whereas Ca2+ cycling and SR Ca2+ leak were measured in Fura-2AM loaded cardiomyocytes. Additionally, t-tubule structure and Ca2+ release synchronicity were measured in single cardiomyocytes.
Results: VF occurred in 8/8 trails in the hearts from HF sedentary, 5/8 trails from HF exercise trained with moderate intensity, 1/9 trails in HF exercise trained with high intensity and 1/13 trails in sham. APD was increased in HF sedentary compared to sham (0.101 ± 0.004 ms vs. 0.093 ± 0.004 ms, respectively). Moderate intensity exercise trained HF had normalized APD compared to sham while high intensity exercise training decreased APD to a level that was shorter than sham (0.085 ± 0.007 ms, $P<0.05$ vs. sham). Currently we are analyzing Ca2+ cycling including SR Ca2+ leak with and without CaMKII inhibitor and PKA inhibitor, AP, t-tubule structure and Ca2+ release synchronicity in single cardiomyocytes which will be finished before the meeting.
Conclusion: High intensity exercise training increase VF threshold and, thus decrease the incidence of ventricular fibrillation in heart failure.

112-Plat

Ca2+ Spark Activity in Intact Dystrophin-Deficient mdx Muscle during Osmotic Challenge is Triggered by Mechanosensitive Pathways

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Inhibitory DHPR control on RyR1 in intact dystrophic mdx skeletal muscle fibres was suggested to be disrupted, resulting in '*uncontrolled*' Ca^{2+} spark frequencies (CSF) during osmotic challenge (Wang et al., 2005, Nat. Cell. Biol.). However, some of their conditions must be considered completely unphysiologic (i.e. 50 mM external Ca^{2+}). We recorded Ca^{2+} sparks in single intact wt, mdx and transgenic mini-dystrophin (MinD) expressing muscle fibres during hypo-/hypertonic challenge using confocal microscopy. CSF were low in wt and MinD, but twofold increased in mdx fibres under isotonic resting conditions. CSF increased faster during hypertonic than hypotonic challenge and peak CSF were about three times larger in mdx vs. wt and MinD fibres. CSF decayed exponentially (τ_{dec}) with ongoing challenge and were significantly faster in mdx fibres, thus questioning '*uncontrolled*' spark activity. In hypertonic solution, CSF τ_{dec} was three times larger when external Ca^{2+} was 50 mM compared to 2 mM. Pretreatment with streptomycine or Gd^{3+} to block mechanosensitive channels (MsC), completely abolished the osmotic CSF increase mdx fibres. Resting membrane potentials in mdx muscle were ~-61 mV and ~-73 mV in wt fibres under hypertonic conditions (2 mM Ca^{2+});

well below thresholds for physiological RyR1 activation. Contributions of MsC to the depolarisation were minor as judged from only slightly more negative resting potentials in the presence of GsMTx4 (-64 mV). We suggest that DHPR inhibition on RyR1 is modulated by MsC in mammalian skeletal muscle and is partly relieved in mdx muscle probably either due to some Ca^{2+} influx through aberrant MsC or direct interactions with the DHPR. A direct DHPR-RyR activation by depolarised membrane potentials in mdx fibers is more unlikely to be a consequence of osmotic membrane stress.

113-Plat

Fluvastatin Alters Both The Calcium Homeostasis And Cell Proliferation In Cultured Myotubes And The Calcium Release Events In Adult Muscle Fibers Of The Rat

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Skeletal muscle cells of newborn rats were cultured in the absence and presence of fluvastatin (0.1 μM). Resting levels of intracellular calcium concentration ($[Ca^{2+}]_i$) were determined from Fura-2 fluorescence while proliferation was assessed by counting the number of nuclei in myogenic cells. The presence of the drug did not alter $[Ca^{2+}]_i$ (130 ± 11 *vs* 121 ± 7 nM; n=12 *vs* 10 rats; mean ± SE; control and treated, respectively) but reduced the number of myogenic nuclei by 50% after 24 hours of treatment. To assess the chronic effects of fluvastatin on skeletal muscles of adult animals, female rats were kept on diets that either contained 62.5 mg/kg (daily intake 6 mg/kg body weight) fluvastatin or not. Animals were either fed with an otherwise normal chow or with a chow that induced an increase in blood cholesterol. Similarly to cultured cells, $[Ca^{2+}]_i$ of adult fibers was unaltered by the drug, however, a clear reduction of muscle mass was observed. Single fibers were enzymatically isolated from the *m. extensor digitorum communis*, permeabilized with Saponin and loaded with Fluo-4. Calcium release events (CRE) were captured using laser scanning confocal microscopy and analyzed with an automated computer program. Fluvastatin increased the frequency of CRE on both normo- and hypercholesterolaemic animals from 0.028 to 0.042 and 0.034 to 0.047 sarc-1s-1 (normo- and hypercholesterolaemic, respectively; n=14 *vs* 14 and 7 *vs* 15). While leaving the full width at half maximum unchanged the drug significantly increased the amplitude of sparks in both groups (0.405 ± 0.005 *vs* 0.436 ± 0.004 and 0.354 ± 0.004 *vs* 0.422 ± 0.005; n=605 *vs* 1429 and 741 *vs* 1052). This gave rise to an increased amount of released calcium in statin treated animals as reflected in the elevated signal mass.

Platform K: Ion Channels, Other

114-Plat

A Microfluidic Approach Enables Ligand Gated Ion Channel Recording from Cell Ensembles

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Electrophysiology remains the preferred method for characterizing ion channel function and kinetics. For screening, it is the most functionally relevant assay, and supersedes flux and fluorescent assays in terms of information content. Many of the medium to high throughput pharmaceutical screens are performed using the 'population patch' approach, which measures current from as many as 64 cells in parallel, eliminating a good deal of the cell-to cell variability of single cell recordings. A major drawback of this method is the inability to exchange solutions during voltage clamp or apply multiple compounds to the same ensemble of cells.

Here we present the first data showing that, by integrating an appropriate microfluidic network design, a large number of cells under voltage clamp can be exposed to a compound within short time scale (50ms) in parallel. A comparison between competing compound injection designs will be presented along with validation data for a number of important ion channel targets. The fluid dynamics of the microfluidic networks were characterized by measuring the time domain response of channel activation and block. Another important aspect that we addressed is the rate of adsorption and desorption of compounds from the channel surfaces. Small fluorescent molecules were used to measure the surface properties as a function of molecular LogP values.

Continuous recording coupled with fast compound additions (50ms for a 20-cell ensemble) opens the way to ensemble recording for ligand gated ion channels, including fast desensitizing channels. We will present ion channel ensemble recording from cell lines expressing GABA-A, P2X3 and TRP-V1 that were obtained using this microfluidic approach.

115-Plat

Discovery Of Photochromic Ligands That Block Voltage-gated K+ Channels At The Internal TEA Binding Site

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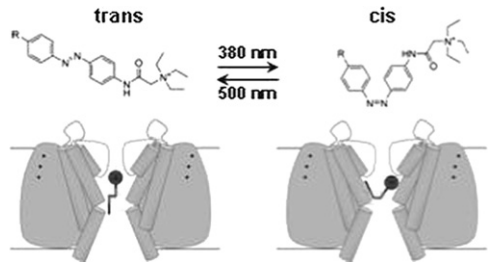
We recently introduced an azobenzene derivative (AAQ) that affords photochemical control of endogenous potassium channels and cellular excitability. This electrophilic molecule was designed to function as a covalently attached photoswitchable affinity label for the external tetraethylammonium (TEA) binding site of potassium channels and was shown to have pronounced and long-lasting effects in various neural tissue preparations. Investigation into the mechanism of action revealed that AAQ is an open-channel blocker of the internal TEA binding site and that covalent modification of the channel is not required to achieve persistent photosensitivity. When applied externally to Shaker IR, AAQ causes fast inactivation in a voltage-dependent manner while exhibiting frequency-dependent block as well as reduced potency when external potassium is increased. Structure-activity studies of analogues lacking electrophilic groups identified blockers with increased potency and revealed structural features that allow the channel to discriminate between isomers. Although most analogues block in the trans form, one analogue (PrAQ) was found to be a cis-blocker. Importantly, the most potent trans-blocker BzAQ was established as an effective substitute for AAQ, affording photocontrol of endogenous K+ channels and action potential firing in dissociated hippocampal neurons.



116-Plat

Gaining control over membrane potential by light using Channelrhodopsin

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Ion channels play a critical roll in many pathological diseases. They can react on very different stimuli like for example ligands, heat, light and voltage. Especially voltage-gated channels are of enormous interest for pharmaceutical companies. Studying those channels in a high-throughput manner is a critical steps towards developing specific drugs.

The light driven cation selectiv ionchannel ChR2 from Chlamydomanas rheinhardtii was already successfully used to depolarized a variety of different neuron cells upon blue light stimulation.

We combined the light guided membran potential change with calcium-sensitive dyes to analyzed the activity of a voltage gated calcium channel. This is also possible because of a very low conductance of calcium ions by light activated ChR2.

Therefore, a stable HEK293 cell line expressing a leak potassium channel (mTrek) and voltage gated calcium channel (CaV3.2) was used. This cell line allows opening of the calcium channel by adding a low KCl concentration in order to activate the CaV3.2 (hyperpolarisation) followed by exchange with a high KCl concentration in order to open the CaV3.2 (depolarisation).

Upon transiently transfecting those cells with ChR2 we made them susceptible to light. In this combination we were able to depolarize the cells with blue light causing an opening of the calcium channel and hence an increased intracellular calcium concentration, which was monitored by fura2. This assay allow us to repeatably open CaV3.2 simply by light.

Also fraction of open channel could be controlled by different light intensity. In a proof of principle experiment the dose-dependent inhibition of know inhibitor could be reproduced. This assay can be easily implemented into a multiwell reader assay and can be used for screening different voltage-gated calcium channels.