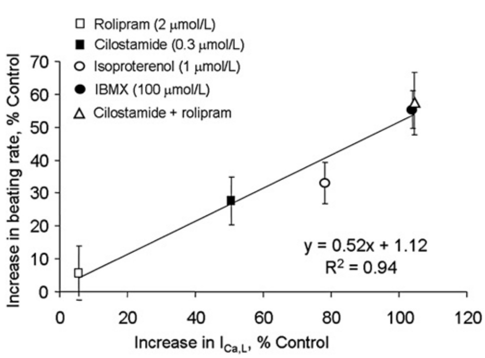
small junctional sarcoplasmic reticulum (SR). With improved resolution and sensitivity that can be achieved by examining Ca^{2+} spark - blink pairs, we report here for the first time small, local but also sometimes spatially extensive Ca^{2+} releases (subsparks and Ca^{2+} "mist") that co-exist with 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)

Tatiana M. Vinogradova, Alexey E. Lyashkov, Harold Spurgeon, Edward G. Lakatta.
NIA, NIH, Baltimore, MD, USA.

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

Sunil Kapur[1], Gary L. Aistrup[1], Rohan Sharma[1], Jiabo Zheng[1], James E. Kelly[1], Alan H. Kadish[1], William Balke[2], J. Andrew Wasserstrom[1].
[1]Northwestern University, Chicago, IL, USA, [2]University of Kentucky, Lexington, KY, USA.

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

Tomas O. Stølen[1], Morten A. Høydal[1], Godfrey L. Smith[2], Ulrik Wisløff[1].
[1]Norwegian University of Science and Technology, Trondheim, Norway,
[2]University of Glasgow, Glasgow, United Kingdom.

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

Martin D.H. Teichmann[1], Frederic von Wegner[1], Rainer H.A. Fink[1], Jeffrey S. Chamberlain[2], Bradley S. Launikonis[3], Boris Martinac[3], **Oliver Friedrich**[1,3].
[1]University of Heidelberg, Institute of Physiology/Pathophysiol, Heidelberg, Germany, [2]University of Washington, Dept. Neurology, Seattle, WA, USA, [3]University of Queensland, Brisbane, Australia.

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+});