We found that an age-dependent reduction in the intrinsic HR in vivo (22%) in
24-vs 3-mo mice is accompanied by a 29% reduction in single SANC AP-firing
rate. These are associated with a number of changes stemming from com-
promised SR Ca$^{2+}$-function, including reduced Ca$^{2+}$-cycling and diminished
response to pharmacologic SR stress. These changes coincide with
decreased expression of crucial SR Ca$^{2+}$-cycling proteins, including SERCA2a
and RyR2, and also NCX1. Aged SANC were also found to have reduced SR
Ca$^{2+}$-load compared to young, as well as a reduced size, number and duration
of spontaneous LCRs. Finally, the sensitivity to PDE inhibition-associated
increase in PLB-photomodulation, LCR size, amplitude, and number were
reduced in 24- vs 3-mo SANC. Thus, a deterioration in intrinsic Ca$^{2+}$-clock
kinetics in aged SANC due to deficits in intrinsic SR Ca$^{2+}$-cycling and its
response to a PDE stress appears to be involved in age-associated
reduction in intrinsic resting AP-firing rate, and intrinsic HR in vivo, and
may also underlie the age-associated reduction in the acceleration of HR
in response to stress.

587-Pos Board B342
Contribution of Ca-Regulated Ion Currents to the Action Potential
Morphology During Cardiac Alternans
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Cardiac alternans, described as periodic beat-to-beat alternations in contrac-
tion, action potential (AP) morphology or cytosolic Ca transient (CaT) ampli-
tude, is recognized as high risk indicator for cardiac arrhythmias, stroke and
sudden cardiac death.
We investigated mechanisms of cardiac alternans in single rabbit atrial myo-
cytes. CaTs were monitored simultaneously with membrane currents or APs
recorded with the patch clamp technique, and revealed a strong correlation
between CaT amplitude and AP duration (APD) during alternans. Investiga-
tion of [Ca]$^{2+}$-Vm interactions indicates that disturbances in Ca signaling are
the primary events mediating activity of Ca-regulated ion channels and lead-
ing to the changes in AP morphology. Voltage-clamp in form of pre-recorded
APs (AP clamp) was used to assess the contribution of Ca-regulated
membrane currents by measuring the difference between currents recorded
during large and small alternans CaTs. Ca-dependent currents exhibited a
large outward component (2.9 ± 0.1 pA/pF) in amplitude) during AP
phases 1 and 2 that was followed by an inward current (0.5 ± 0.1 pA/pF in
amplitude; n=7) during AP repolarization. We also investigated contributions of Na/Ca exchange (NCX), L-type Ca, Ca-activated K and small
current-conductance Ca-activated K currents to the AP morphology during
Ca alternans. ~90 % of the initial outward current was blocked by substitution
of Cl ions or application of Cl channel blocker (DIDS) indicating that this
current is mainly carried by Ca-activated Cl channels. In summary, during
Ca alternans the prominent prolongation of AP at APD10 to APD30 re-
polarization levels was due to reduced Ca-activated Cl current while the overall effect of other Ca-sensitive currents (NCX, L-type Ca,
small conductance K) cause slight shortening of AP in the APD50 to
APD90 range.

588-Pos Board B343
A Small Number of Cells is Sufficient to Trigger a Cardiac Arrhythmia:
Stochastic Computational Studies
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Cardiovascular disease is the leading cause of death world-wide and this is
due in large part to arrhythmias. Here we examine the cellular and subcellular
basis of Ca$^{2+}$ dependent arrhythmias. In order to understand how calcium
dynamics, plays a role in arrhythmogenesis, we have investigated normal and
dysfunctional Ca$^{2+}$ signaling in heart cells at high temporal and spatial
resolution. Spontaneous calcium release occurs normally as Ca$^{2+}$ sparks.
When RyR2 open probability increases to a high level, then Ca$^{2+}$ waves
can activate Ca$^{2+}$ waves. These propagating elevations of [Ca$^{2+}$]c can acti-
vate inward NCX current (INCX ) that may contribute to early after-
depolarization (EADs) and underlie delayed after-depolarization (DADs).
However, how cellular currents lead to full depolarizations of the myocard-
dium, and how they initiate extra systoles is still not fully understood.
Some earlier studies that have investigated this question suggest that as
many as about ~700,000 cells must undergo such behavior to initiate a prop-
gating action potential or an arrhythmia. Here we present the results of our
study which explores how many cells must be entrained to initiate arrhythmo-
genic depolarizations in “realistic” computational models. The model pre-
sented here suggests that only a small number cells must activate in
order to trigger an arrhythmogenic propagating action potential. These condi-
tions were examined in 1D, 2D, and 3D taking into account heart geometry.
The finding that only a small number of cells is required to trigger an
arrhythmia provides a plausible mechanism by which cardiac arrhythmias
might occur.

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Relative Contribution of Purkinje Fibers to Ca$^{2+}$-Dependent Arrhythmias in a Murine Model of CPVT
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Background. Purkinje fibers (PFs) are critical for coordinated electrical exci-
tation of the ventricles and thought to play a key role in abnormal impulse
formation and ventricular arrhythmias. However, the arrhythmogenic prop-
terties of PFs as compared to ventricular tissue remain to be elucidated and
were the focus of the present study.
Results. To examine the arrhythmogenic potential of PFs vs ventricular tissue we
performed Ca$^{2+}$ and membrane potential (MP) imaging in PFs and ventricu-
lar tissue preparations derived from both wild-type (WT) mice and
genetically modified mice prone to Catecholaminergic Polymorphic Ventricular
Tachycardia (CPVT). PFs and trabecula dissected from the right ventricles were loaded with the voltage- and Ca$^{2+}$-sensing dyes RH237 and
Rhod-2, respectively. PFs were characterized by the narrow shape and lack of T-tubules of Purkinje cells, as evidenced by membrane
staining with RH237. In PFs and trabecular preparations isolated from
CPVT but not WT mice, exposure to the beta-adrenergic agonist isoprote-
renol resulted in frequent diastolic Ca$^{2+}$ releases both spontaneous and trig-
gered, that were associated with corresponding MP signals. Consistent with their ability to lead to triggered events, diastolic spontaneous Ca$^{2+}$ releases
showed high synchronicity between adjacent cells in both tissues. Overall
spontaneous Ca$^{2+}$ release synchronicity and the rates of occurrence of spon-
taneous and triggered Ca$^{2+}$ release events were similar between PFs and
 trabeculae.
Conclusion. These results suggest that the Purkinje system and ventricular
tissue possess similar arrhythmic potentials in a setting of CPVT, consistent
with a diffuse nature of the genesis of Ca$^{2+}$-dependent ventricular arrhythmias.

590-Pos Board B345
The Metabolic Modulator Perhexiline Induces Calcium-Cycling Dysfunction and Apoptosis in Cardiomyocyte Syngenic C57BL/6 J Mice
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Major pathogenic adaptations in chronic heart failure (CHF) include the
reversion to glycolytic metabolism and the persistence of an energy deficient state. The use of metabolic modulators to increase the efficiency of glycolytic ATP
production has emerged as a new therapeutic approach in CHF. Perhexiline
(PHX), an anti-anginal agent, improves myocardial function in CHF patients
by a mechanism that is thought to involve the inhibition of carnitine palmitoyl
transferase (CPT) which prevents mitochondrial free fatty acid uptake. In
silico modelling and cell-based experimental data predict that such alterations
to the metabolic steady state would drive other compensatory (mal)adapta-
tions that could negatively impact on cellular phenotype. Here, we investi-
gated the phenotypic consequences of PHX-mediated metabolic modulation
on Ca$^{2+}$ cycling and apoptotic susceptibility in HL-1 syngentic. HL-1 cardio-
myocytes exhibit a metabolic profile similar to that observed in CHF myocardium
(glycolysis >> fatty acid oxidation). Contrary to the expected enhancement of Ca$^{2+}$ cycling, PHX profoundly downregulated Ca$^{2+}$ signalling in a
time- and concentration-dependent manner. The spatio-temporal organiza-
tion and intercellular synchronization of Ca$^{2+}$ oscillations was progressively
reduced at [PHX] between 0.1 and 2.5μM and contractility was abolished at
[PHX] > 5μM. These concentrations are below the levels of drug reported
to accumulate in heart tissue following therapeutic dosing. Metabolic activity
was compromised in PHX-treated cells and we determined PHX
concentration-dependent caspase-linked apoptosis (EC50 ≈ 25μM). Oxen-
ficine (a potent CPT1 inhibitor) did not recapitulate any of these effects at
carboxylations up to 100μM and cell data show that, in the context of glycolytic
metabolism, PHX provoked dysfunctional Ca$^{2+}$ handling and increased cardi-
omyocyte susceptibility to apoptosis. These effects appeared to be indepen-
dent of CPT inhibition.