mathematical modelling to tease out changes in cell characteristics responsible for the differences in calcium and contraction profiles between load-free and after-load environment.
Mathematical Method: We coupled the Shannon-Bers ventricular action potential model to a viscoelastic model to simulate the myocyte contracting in either a load-free condition (Tyrode solution) or under load (in the gel matrix). The mathematical model establishes a closed feedback loop between the calcium system and the extracellular environment that gives rise to the self-regulation we observed. We ran extensive simulations where parameters associated with the influx and efflux of Ca^{2+} were modulated such that they are either up-regulated or down-regulated by NO. In silico results are filtered out to qualitatively match cell-in-gel in vitro results. The filtering process is based on measures that capture multiple properties of calcium profiles.
Conclusion: Our approach of identification hints that the upregulation of NO has the effect of simultaneously modulating multiple parameters of the Ca^{2+} handling pathway. Of the modulated parameters, the L-type current amplitude has to be consistently increased. Coupled with the increase in L-type current, parameters associated with release and uptake of calcium by the SR have to be modulated in opposite directions.

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Pharmacological Targeting of Serca May Have Potential for Cellular Protection
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We previously reported that CGP-37157 and K201, two benzothiazepines (BZT) with cardioprotective action, inhibit the Ca^{2+} ATPase of sarcoplasmic reticulum (SR) intracellular Ca^{2+} stores (SERCA). We tested if SERCA block could be part of the mechanism by which drugs protect cells from ischemic damage. We also screened structural characteristics in BZT that could affect their drug potency as SERCA inhibitors. SR microsomes isolated from rabbit skeletal muscle (SkM) and pig heart ventricle were utilized to measure modulation of SR Ca^{2+} loading and SERCA-mediated ATPase activity by drugs. Seven out of twenty cell-protective drugs tested (including pimozide, EGCG, KN-93 and carvedilol) inhibited, at least partially, SR Ca^{2+} loading and Ca^{2+}-stimulated ATPase activity in SkM and heart SR microsomes. We also screened ten novel BZT derivatives and seven FDA-approved benzodiazepines (BZD; including bromazepam and clonazepam), which have close homology to CGP37157. Ca^{2+}-dependent block of SERCA was found in four BZT's, which displayed higher (PH000995, PH000902) and similar potency (PH000902, PH006796) compared to CGP. BZD were all ineffective, which suggest that the sulfur atom in the BZT ring (substituted by nitrogen in BZD) is crucial for their SERCA blocking ability. All compounds above, were tested on ryanodine receptor (RyR) activity (planar bilayers, SR leak, [^{3}H] ryanodine binding). None of these agents directly inhibited RyR function in heart and muscle. In contrast, some agents (BZT and BZD) had mild agonistic action on channel function. We think that SERCA block by these drugs, which persists at pH ~6.5, may benefit ischemic cells by preventing SR Ca^{2+} overload, known to trigger, upon reperfusion, abnormal RyR-mediated Ca^{2+} leak associated with cell death and tissue injury. BZT have potential as templates for therapeutic targeting of SERCA (Supported by AHA and Eskridge Foundation).

2313-Pos Board B329
The Interplay between FGF23- and Angiotensin II- Mediated Calcium Signaling in Cardiac Hypertrophy
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Fibroblast growth factor 23 (FGF23) is a hormone strongly linked to heart failure and cardiovascular mortality. It triggers pathological Ca^{2+}-regulated transcriptional pathways leading to left ventricular hypertrophy. *In vivo*, high circulating levels of FGF23 are associated with an altered renin-angiotensin-aldosterone system response. Here we investigated Ca^{2+}-dependent signaling of FGF23 and its interconnection with angiotensin II (ATII) in neonatal rat ventricular myocytes (NRVMs). Both ATII and FGF23 induced hypertrophy in NRVMs as reflected by cell area and hypertrophic gene expression. In Ca^{2+} imaging experiments, an increase of cytoplasmic (2.4folds ± 0.3) and nuclear (1.9folds ± 0.3) Ca^{2+} transient amplitude was observed on acute treatment with FGF23 ($p \leq 0.01$) similar to ATII. CaT integral too was augmented significantly by both the treatments in cytoplasm and nucleus. A selective pro-hypertrophic enhancement of nuclear Ca^{2+} release as seen in ATII treatment was evident in FGF23-treated NRVMs (1.8folds ± 0.2) when the nuclear integral was normalized to the corresponding cytoplasmic integral (ratio). Localised nuclear Ca^{2+} release involves agonist (ATII)-led generation of inositol trisphosphate (IP3) and stimulation of nucleolemmal IP3-receptor (IP3R). IP3R inhibitor reverted the effect of FGF23($p \leq 0.01$) on integral ratio implying the involvement of IP3 in the FGF23-mediated prolonged nuclear Ca^{2+} release. Our results reveal comparable response of NRVMs to FGF23 and ATII at multiple levels suggesting a crosstalk between their signaling. Interestingly, ATII receptor antagonist significantly attenuated FGF23-induced hypertrophy and changes in Ca^{2+} homeostasis. Long, as well as acute application of FGF23, increases intracellular expression of ATII peptide (2.2folds ± 0.1) vs. untreated NRVMs in a time-dependent manner, confirming ATII contribution. Nevertheless, results of ongoing mass spectrometry profiling of FGF23-induced intracellular and secreted ATII in NRVM culture will be presented in detail. In conclusion, FGF23 may lead to a pathological activation of ATII signaling, which contributes to hypertrophy in cardiomyocytes.

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Calmodulin Mutations Associated with Congenital Cardiac Disease Display Novel Biophysical and Biochemical Characteristics
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Calmodulin (CaM) is a cytoplasmic multifunctional calcium (Ca^{2+})-binding messenger that interacts with the cardiac ryanodine receptor (RyR2), a large transmembrane Ca^{2+} channel that mediates Ca^{2+} release from the sarcoplasmic reticulum (SR) to activate cardiac muscle contraction. Recent genetic studies have reported CaM missense mutations in patients with a history of severe cardiac arrhythmogenic disorders. Herein, we have investigated the effect of four novel missense CaM mutations, identified in two patients presenting with long QT syndrome (LQTS) (N98I, D134H), and two patients with clinical features of both LQTS and catecholaminergic polymorphic ventricular tachycardia (CPVT), (D132E and Q136P), relative to the biophysical and biochemical properties of wild type CaM (CaM^{WT}). We used CD spectroscopy to examine the thermal stability of CaM^{WT} and mutant proteins. In the absence of Ca^{2+}, thermodynamic values for all proteins were similar. In contrast, in the presence of Ca^{2+}, there was a significant decrease in the stability of the five proteins following the order $CaM^{WT} > CaM^{N98I} > CaM^{D132E} > CaM^{Q136P} > CaM^{D134H}$. Further Ca^{2+}-binding studies revealed that all CaM mutations significantly reduce the Ca^{2+}-binding affinity of CaM^{WT}. CaM^{Q136P} protein exhibited a ~7-fold reduced Ca^{2+}-binding affinity compared to CaM^{WT}, while CaM^{D132E} had a ~14-fold reduction. Furthermore, biochemical analysis revealed that all four CaM mutants displayed dramatically reduced RyR2 interaction and defective modulation of [^{3}H]ryanodine binding to RyR2, regardless of LQTS or CPVT association. Our findings confirm our previous observations suggesting that the clinical presentation of LQTS or CPVT associated with these four CaM mutations may involve both altered intrinsic Ca^{2+}-binding as well as dysregulation of RyR2-mediated Ca^{2+} release via aberrant interaction of CaM with RyR2.

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Dystonia-Associated Hippocalcin Mutants Dysregulate Cellular Calcium Influx
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Dystonia is a neurological movement disorder that provokes muscle spasms and contractions. It is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements and painful postures. Recently, mutations at positions T71N and A190T in the neuronal calcium-binding protein hippocalcin, have been shown to be critical in development of DYT2 dystonia. However, the effect of these mutations on the physiological role of hippocalcin has not yet been elucidated. Using a multidisciplinary approach, we showed that mutations T71N and A190T in hippocalcin did not affect stability, calcium-binding affinity, translocation to cellular membranes