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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.

2312-Pos Board B328
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.

2314-Pos Board B330
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.

2315-Pos Board B331
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

(Ca^{2+}/myristoyl switch) and three-dimensional structure of the protein. However, the disease-associated mutations caused a defect in calcium-induced oligomerisation of hippocalcin. In KCl-stimulated SH-SY5Y cells expressing mutated hippocalcin, we observed an increased calcium influx, mostly driven by N-type voltage-gated calcium channels. Our data demonstrate that the dystonia-associated mutations strongly affect hippocalcin cellular functions which suggest a central role for perturbed calcium signalling in DYT2 dystonia.
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2316-Pos Board B332
Role of NAADP for Calcium Signaling in the Salivary Gland
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The secretion of saliva in response to physiological demand is under control of the autonomic nervous system. Coordination and regulation of intracellular Ca^{2+} signaling following autonomic activation is crucial for proper salivary secretion. Previous work from our lab has demonstrated parotid acinar cells contain an abundant and polarized distribution of acidic organelles. Furthermore, acidic organelles are recruited for agonist-induced Ca^{2+} signaling following cAMP elevation. The current study interrogated a role for NAADP, a potent second messenger known to release Ca^{2+} from acidic organelles. In parotid acinar cells, initial results demonstrated NED19, an inherently fluorescent NAADP receptor inhibitor, was localized to acidic endosomes. Additionally, using a whole-cell patch clamp method to dialyze NAADP into parotid acini resulted in robust Ca^{2+} -activated Cl^-currents. Peak activation occurred at low nanomolar concentrations of NAADP and higher concentrations showed an inhibitory effect. These observations suggest a role for NAADP in producing the fluid component of saliva. Thus, this work identifies a NAADP signaling pathway as a potential therapeutic target for people suffering from salivary hypofunction.

2317-Pos Board B333
Spontaneous Ca^{2+} Fluctuations Mediated by TRPM7 Channels in Growth Plate Chondrocytes
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During embryonic bone outgrowth, round chondrocytes intensively proliferate and then sequentially differentiate into columnar and hypertrophic chondrocytes in the growth plate cartilage. These processes might be regulated by intracellular Ca^{2+} signaling, however Ca^{2+} handling has been poorly explored in growth plate chondrocytes. We currently developed an experimental method for fluorometric Ca^{2+} imaging of intact chondrocytes in bone slice preparations from E17.5 mouse embryos. In this imaging system, we found spontaneous intracellular Ca^{2+} fluctuations, characterized by small peak amplitudes ranging in estimated Ca^{2+} concentration of ~50 nM, in round and columnar chondrocytes. The spontaneous Ca^{2+} fluctuations were attenuated by FTY720 and NS8593, both of which inhibit the transient receptor potential melastatin subfamily 7 (TRPM7) channel, a non-selective cation channel that can conduct Ca^{2+} and Mg^{2+}. In contrast, the TRPM7 activators naltriben and NNC550396 facilitated the Ca^{2+} fluctuations. In accordance with these modulator effects, microarray analysis suggested that *Trpm7* is expressed in growth plate chondrocytes. Furthermore, the phospholipase C (PLC) inhibitor U73122 depressed the Ca^{2+} fluctuations, while the large-conductance calcium-activated-K^+ (BK) channel activator NS1619 stimulated them. Therefore, growth plate chondrocytes generate spontaneous intracellular Ca^{2+} fluctuations, which are likely mediated by TRPM7 channels and maintained by PLC and BK channel activities. We now attempt to address the role of TRPM7-mediated Ca^{2+} fluctuations in bone development.

2318-Pos Board B334
Calmodulin Interaction with Gap Junction Intracellular Loop Peptides
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Connexins (Cx) are membrane-spanning proteins that form gap junctions which allow the exchange of small molecules between cells, e.g. ATP, IP_3 and ions. Although calmodulin (CaM) binding sites have been identified in three regions, the N- and C-terminal tails[1,2] and the intracellular loop (ICL)[3], moreover CaM blocks Cx45 gap junctions[4], the role of CaM in gap junction function is not well understood. Thus, CaM interaction with synthetic peptides of ICL from representatives of each the three Cx subgroups was investigated. Fluorescence changes of double labelled FRET-probe DA-CaM and Ca^{2+} sensitive TA-CaM were monitored by fluorescence spectroscopy and stopped-flow fluorimetry at physiological ionic strength and pH 7.5 and 20 °C. Both Ca^{2+}-dependent and -independent interactions were identified. K_d values of 40 ± 4 nM, 31 ± 3 nM, 75 ± 4 nM and 60 ± 7 nM in Ca^{2+} and 924 ± 223 nM, 3.2 ± 0.5 µM, 849 ± 105 nM and 625 ± 123 nM in the absence of Ca^{2+} were obtained for ICL of Cx32, Cx35, Cx45, Cx57, respectively. FRET measurements revealed partial compaction of DA-CaM (54-70% quenching in the presence of Ca^{2+} and 33-62% quenching in the absence of Ca^{2+}). The kinetic data revealed a two-step process of rapid binding followed by isomerisation. Evidence for both Ca^{2+}-dependent and independent binding indicates that CaM may be anchored to gap junctions in resting cells and becomes fully bound upon stimulation. Thus, our data strongly suggest a modulatory role for CaM-ICL interactions in gap junction regulation.
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2319-Pos Board B335
Mathematical Modeling of Calcium Signaling in Microglia
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Microglia function is orchestrated through highly-coupled signaling pathways that depend on calcium. In response to external stimuli, microglia commonly present elevated intracellular calcium (Ca^{2+}), stemming from either external sources, e.g. through plasma membrane Ca^{2+} channels, or internal sources, including IP3 mediated endoplasmic reticulum Ca^{2+} release. Numerical models of Ca^{2+} -signaling processes in microglia could provide a basis for interpreting wide-ranging experiments and offering testable hypotheses. Here, we have created a computational model of microglial Ca^{2+} handling, including ATP-dependent P2X activation, p38/ERK kinase activity, activation of NFAT transcription factors, as well as tumor necrosis factor alpha secretion. The model was trained using published data for each of these processes. With this model, we have probed the extent to which triggering of Ca^{2+} influx via activation of plasma membrane Ca^{2+} channels, namely P2X channels, controls intracellular Ca^{2+} , and its propensity to influence transcription. Given that Ca^{2+} dysregulation in microglia is common to many neurological diseases, we anticipate that our model may provide a framework to better understand and probe microglial pathophysiology.

2320-Pos Board B336
Effect of Calcium Flux on Filopodia of Epithelial Cells
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Filopodia are the sensors of a cell and are responsible for directing cell motility. Like ''antennae,'' they receive signals from both soluble ligands in the extracellular mileau and those attached to the substrate. In the nerve axon, these two aspects of signaling are integrated so as to modulate chemotactic signaling according to the composition of the substrate. The role of filopodia in the modulation is not clear. Although epithelial cells differ from nerve cells in their organization, the same second messengers mediate the filopodia's sensory function. In both cell types, activation of a PKC (protein kinase C) with a tumor promoter inhibited filopodia or caused their dissolution. Because Ca^{++} selectively activates one subset of PKCs, local Ca^{++} elevation could activate these isozymes and also thereby decrease filopodia. Previous reports showed contradictory results for Ca^{++} second messenger, however, in the axon, i.e. positive or negative effects on filopodia. Here, we investigate how filopodia dynamics are affected by calcium. We measured percentage of the cell periphery covered with filopodia and percentage of cells showing filopodia. There is an inhibitor of filopodia in culture media, so that replacing the medium with a buffer increased filopodia. This occurred regardless of whether calcium was present in the buffer or absent. When cells remained in the medium, however, inhibitors of calcium transport caused a decrease in filopodia. Cyclopiazonic acid in Ca^{++}-free buffer typically blocks uptake of Ca^{++} into the endoplasmic reticulum (ER) and causes net Ca^{++} efflux. This enhanced filopodia. Restoring extracellular Ca^{++} in the extracellular buffer after depleting the ER store also enhanced filopodia production. We conclude that filopodia display was sensitive to calcium flux but not to absolute physiologically relevant calcium concentrations. It remains to be determined how calcium flux is detected in epithelial cells.