the walls of hollow organs. Increase in cytosolic [Ca^{2+}] results from agonists released by nerve terminals and binding ionotropic membrane receptors thereby opening Ca^{2+} channels or metabotropic receptors thus activating Ca^{2+}-mobilization through secondary messengers. Extra [Ca^{2+}] in the proximity of ER ryanodine receptors stimulates Ca^{2+}-induced Ca^{2+}-release from ER stores that is inhibited over certain [Ca^{2+}]. The model considers also regenerative ICR propagation through agonist-stimulated-agonist-release by certain hemichannels activated directly by agonists or through activation of certain purinergic receptors. Degradation of bound agonists together with receptor desensitization progressively attenuate both Ca^{2+} influx and Ca^{2+} release from the ER stores. Basal [Ca^{2+}] is then restored by Ca^{2+}extrusion pumps and by resequestering excessive Ca^{2+} within ER stores and mitochondria. The event sequence propagates to neighboring cells by diffusion of agonists through extracellular medium and of Ca^{2+} and second messengers through gap junction channels between coupled cells. Variability of ICS is provided by randomly distributing the myocytes with a given density in a 3D staggered grid and randomly selecting from experimentally determined intervals values characterizing the extracellular compartment, individual cells, cell interconnections and receptor activation and desensitization in healthy and diseased humans and animal models of human diseases. The model allows us to mimic consequences of alterations of individual and combined ICS components to better understand the etiology of abnormal smooth muscular activity and to explore limitless therapeutic avenues of diseases with deleterious effects on the smooth musculature. By determining the statistics of large libraries of simulations, we can evaluate the incidence of various phenotypes.

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Increased Sensitivity to Ischemia in an Early Diabetic Cardiomyopathy: The Role of Calcium Handling

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Diabetic cardiomyopathy is characterized by early-onset diastolic dysfunction and late-onset systolic dysfunction. However, little is known about the mechanisms underlying the response of the diabetic myocardium to ischemia.

Aim - To study the left ventricular (LV) dysfunction and the role of calcium handling in infarcted diabetic mice in an early stage of diabetic cardiomyopathy.

Methods and Results - A cohort of male diabetic *db/db* and age-matched non-diabetic control mice at 10 wk of age was randomly assigned into Sham and myocardial infarction (MI) groups. MI was induced by coronary ligation. Standard echocardiography and tissue Doppler imaging were performed by high-resolution in-vivo imaging system, and diastolic sarcoplasmic reticulum (SR) calcium leak was measured in isolated cardiomyocytes using fluorescence microscope. One month after MI, 75% of the nondiabetic mice survived vs. 55% of the MI diabetic mice ($p=0.04$). A significant LV dilatation was observed in MI diabetic mice compared to nondiabetic ($p=0.03$). Peak systolic tissue velocity (Sm) was 28% lower in MI diabetic mice than in nondiabetic group (non-diabetic: 19 ± 1 vs. 17 ± 2mm/s; diabetic: 18 ± 2 vs. 13 ± 2*mm/s, *$p=0.05$, for Sham and MI, respectively). Peak early diastolic tissue velocity (Em) was decreased in both Sham and MI diabetic mice (17 ± 2* and 16 ± 4* vs. 25 ± 3 and 25 ± 4 mm/s, *$p<0.05$, respectively). Diastolic SR calcium leak was unchanged in 10-wk diabetic mice compared with nondiabetic mice. However, a significant increase diastolic SR calcium leak was observed in MI diabetic mice relative to MI nondiabetic mice.

Conclusion - The altered calcium homeostasis is an important determinant of sensitivity to ischemia and of loss of the ventricular function in the early stage of diabetic heart disease in diabetic mice.

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Calcium Induced Conformational Changes In The Cytoplasmic Tail Of Polycystin-2

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Autosomal dominant polycystic kidney disease (PKD) is the most common, monogenic cause of kidney failure in humans, characterized by the presence of fluid filled cysts in kidneys, liver, pancreas and intestines. Most cases of PKD are linked with mutations in the genes *Pkd1* or *Pkd2*, which encode proteins polycystin-1 (PC1) and polycystin-2 (PC2) respectively. Here we focus on PC2 a calcium (Ca^{2+}) permeable channel in the transient receptor potential (TRP) channel family. PC1 and PC2 interact directly and this interaction is thought to be mediated by their cytoplasmic carboxyl terminal tails. The deletion of the carboxyl terminus of either PC1 or PC2 alters Ca^{2+} signaling; the most common pathogenic mutations in PC2 are premature truncations. We have previously identified two stable domains within the C-terminus of PC2. The first is an EF-hand Ca^{2+} binding domain. The second is a previously unreported coiled-coil domain which we show is responsible for oligomerization of PC2 using Small-Angle X-ray Scattering (SAXS), Analytical Ultracentrifugation and Size Exclusion Chromatography. We show by Isothermal Titation Calorimetry that the EF-hand domain binds Ca^{2+} and that mutations in the predicted Ca^{2+} binding loop abolish the affinity for Ca^{2+}. We hypothesize that the EF-hand domain serves as a Ca^{2+} sensor/switch, and show that PC2 undergoes Ca^{2+} induced conformational changes by NMR, CD, and SAXS. We have completed NMR experiments necessary for high resolution structure determination in the presence and absence of Ca^{2+} and have identified PC2 residues with significant chemical shift changes upon Ca^{2+} titration. We have obtained crystals of the coiled coil domain of PC2 and are optimizing crystals for X-ray diffraction. Structure determination of these cytoplasmic domains will enable a structure-guided analysis of PC2 mediated Ca^{2+} signaling and further investigations into the molecular basis of PKD progression.

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L163,255, a Synthetic Growth Hormone Secretagogue, Raises [Ca^{2+}]$_i$ by Promoting Intracellular Calcium Stores Depletion in Intact Fast-Twitch Fibers of Rat Skeletal Muscle

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The synthetic growth hormone secretagogues, GHS, and the endogenous ghrelin are small molecules proposed as pharmacological tools for the treatment of GH deficiency conditions in view of their ability to stimulate the GH release. Other than in pituitary gland, GHS receptor binding sites are documented in peripheral tissues accounting for a series of GHS pleiotropic activities. Accordingly, a direct action of GHS on skeletal muscle has been proposed, as they reduced resting chloride and potassium conductances in muscle fibres, probably through the activation of a GHS-receptor linked to PLC/PKC/Ca^{2+} signalling (Pierno et al. 2003. *Br. J. Pharmacol*. 139:575–584). By using fura-2 fluorescent measurements, we evaluated the effect of L163,255, a non peptidic GHS, on calcium homeostasis of rat *Extensor Digitorum Longus* (EDL) fibers mechanically isolated. *In vitro* application of L163,255 increased cytosolic calcium concentration, [Ca^{2+}]$_i$, in a dose-dependent manner with an IC_{50} of ~ 300 μM. Particularly, application of 200μM L163,255 led to [Ca^{2+}]$_i$ increase from 26 ± 2 nM to 164 ± 34 nM after 10 min of incubation.Removal of external Ca^{2+} in the bath solution did not abolish L163,255 effects. On the contrary, pre-incubation with the Ca^{2+}-ATPase inhibitor thapsigargin or the mitochondrial permability transition pore inhibitor Cyclosporin A partially and strongly reduced L163,255-induced Ca^{2+} transient respectively, suggesting the involvement of thapsigargin-sensitive calcium stores and mitochondria in the drug action. These data support the presence of GHS receptors on skeletal muscle and highlight that GHS, proposed as therapeutic drugs, by altering calcium homeostasis could interfere with skeletal muscle functionality.

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Multiple Sources of Light-Evoked Intracellular Calcium Increases in *Hermissenda* Type B Photoreceptors

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Previous research suggests that learning-produced changes in excitability and K^+ currents of *Hermissenda* Type B photoreceptors are Ca^{2+}-dependent phenomena. Little information is available concerning the sources and dynamics of Ca^{2+} in these cells. We have used Fura-2 dual-wavelength (340/380 nm) photometry to measure somatic [Ca^{2+}]$_i$ in B cells. Thirty sec light steps (LS) produce a large increase in [Ca^{2+}]$_i$ (~ 246%). To determine the contribution of Ca^{2+}-influx vs Ca^{2+}-release, we measured [Ca^{2+}]$_i$ throughout 5 consecutive LSs in either normal or Ca^{2+}-free ASW (0 mM Ca^{2+}, 30 mM EGTA). Cells exposed to Ca^{2+}-free ASW had a basal [Ca^{2+}]$_i$ much lower than when external Ca^{2+} was present, often below detection limits. Ca^{2+}-free ASW abolished light-induced [Ca^{2+}]$_i$ increases in all 7 cells tested. We next explored the role of voltage-gated Ca^{2+} channels (VGCCs) to Ca^{2+} influx with the use of cobalt (5mM), a VGCC blocker in B cells. Co^{2+} did not affect either basal [Ca^{2+}]$_i$ or light-induced [Ca^{2+}]$_i$ increases ($n = 5$). To assess the contributions of the ER to light-induced [Ca^{2+}]$_i$ changes, B cells were incubated in the ryanodine receptor (RyR) blocker dantrolene (50 μM). Dantrolene reduced the [Ca^{2+}]$_i$ response by ~33% ($n = 5$), and also produced a progressive reduction in basal [Ca^{2+}]$_i$ (~ 60%). Exposure of Ca^{2+}-free ASW cells ($n = 3$) to thapsigargin (TH; 100μM - 1mM, a blocker of the ER Ca^{2+}-ATPase pump) increased basal [Ca^{2+}]$_i$, consistent with store depletion. Collectively, our results indicate that [Ca^{2+}]$_o$ is necessary for normal basal [Ca^{2+}]$_i$ and critical for light-induced [Ca^{2+}]$_i$ increases; but little Ca^{2+} enters through VDCCs. This suggests that [Ca^{2+}]$_o$ enters the cytosol via other routes (e.g. TRP channels) or that the contribution of Ca^{2+} through VGCCs is slight but serves to trigger Ca^{2+}-induced Ca^{2+}-release (CICR) from ER stores.