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Intracellular Free Zinc Ion Increase Triggers Hyperglycemia-Induced Cardiomyocyte Dysfunction through Endoplasmic Reticulum Stress

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Intracellular free Zn^{2+} concentration, $(Zn^{2+})_i$ is less than one nanomolar in resting cardiomyocytes. Very little is known about mechanisms controlling $(Zn^{2+})_i$ distribution and variations during cardiac function although *in vitro* oxidant exposures to cardiomyocytes caused 30-fold increase in $(Zn^{2+})_i$ but only 2-fold in $(Ca^{2+})_i$ while these were 2-fold and 1.2-fold under hyperglycemia, respectively. Since it has been mentioned that Zn^{2+} homeostasis is involved in unfolded protein response (endoplasmic reticulum stress, ERS) under salt stress, we aimed to investigate whether a hyperglycemia-induced increased level of basal $(Zn^{2+})_i$ can trigger an ERS, which in turn induces marked cardiomyocyte dysfunction. When we enforced Zn^{2+} influx into cytosol by using zinc ionophore pyrithione (ZnPT), mechanical and electrical activities of cardiomyocytes impaired in a concentration-dependent manner, similar to hyperglycemia. Incubation of cardiomyocytes with ZnPT exerted also hyperphosphorylation in RyR2, PKA, and CaMKII in a concentration-dependent manner, again similar to hyperglycemia. The levels of some ERS chaperon proteins and ERS-mediated apoptosis marker proteins (Calregulin, GRP 78, Bcl-2 and PUMA) were also examined and compared in between ZnPT-incubated cardiomyocytes and STZ-diabetic rat cardiomyocytes. The chaperon protein levels were found to be increased in diabetics and also in ZnPT-incubated ones in a concentration dependent-manner. We also observed a similar parallel correlation between these two-group experiments for anti-apoptotic protein Bcl-2 and apoptotic one PUMA. Taken into consideration diabetes-induced cardiac dysfunction due to, in part, increased basal $(Zn^{2+})_i$ and *in vitro* increased $(Zn^{2+})_i$-induced cardiomyocyte dysfunction present a possible triggering role of increased $(Zn^{2+})_i$ in hyperglycemia-induced cardiomyocyte dysfunction through ERS.

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K^+ Channel-Interacting Protein 2 Deficient mice have a Rate Dependent Prolongation of Left Ventricular CA^{2+} Transients

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In the heart, K^+ channel-interacting protein 2 (KChIP2) stabilizes Kv4 channels in the membrane resulting in larger transient outward potassium currents (I_{to}). Recently it has been demonstrated that KChIP2 also enhances Cav1.2 and that the KChIP2$^{-/-}$ mouse has reduced inward L-type Ca^{2+} current. In this study, we test the impact of KChIP2$^{-/-}$ on cardiac contraction and on underlying Ca^{2+} transients.

Through ultrasound examination, we found comparable left ventricular ejection fraction and fractional shortening in the heart of wild-type (WT) and KChIP2$^{-/-}$ mice, indicating that the function of the left ventricle was not impaired despite reduced L-type Ca^{2+} current. Confocal line scan Ca^{2+} imaging was performed using the Cal-520^{TM} fluorophore. Whole-cell Ca^{2+} transients were recorded from disaggregated left ventricular cardiomyocytes at 37°C, field stimulated at cycle lengths between 80 and 1000 ms. A decrease in the relative peak of the Ca^{2+} transient was found in KChIP2$^{-/-}$ versus WT (0.58 ± 0.06 vs. 0.71 ± 0.07 F/F_0, $P<0.05$) and the time to 50% decay of the transient was prolonged (133 ± 5 vs. 121 ± 3 ms, $P<0.05$). These differences were rate-dependent and diminished at cycle lengths shorter than 1000 ms. However, at the fastest pacing rate more cardiomyocytes were alternating in KChIP2$^{-/-}$ compared with WT (cycle length: 80 ms; WT: 12/18, KChIP2$^{-/-}$: 23/23). The fractional Ca^{2+} release was not different between the two genotypes, suggesting that the differences in Ca^{2+} transients are not a result of differential SR load.

In conclusion, although cardiac contraction is not impaired in the KChIP2$^{-/-}$ mouse, some abnormal Ca^{2+} handling is apparent in individual KChIP2$^{-/-}$ cardiomyocytes, including a higher propensity to develop Ca^{2+} transient alternans. If this is manifested significantly at the tissue level, it could contribute to the development of repolarization gradients across the myocardium.

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Calcium Handling in Experimental Models of Doxorubicin and Radiation-Induced Cardiotoxicity

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As a result of more effective therapies, more patients recover from cancer. However, often they develop cardiotoxicity manifested, among others, as heart failure. Doxorubicin (Dox), a widely used and highly effective cancer chemotherapeutic agent, and radiation therapy (RT), when directed to the thorax, can affect cardiac function. Because intracellular Ca^{2+} fluxes underlie cardiac function, we investigated intracellular Ca^{2+} handling in mice treated with doxorubicin (Dox) or chest radiotherapy (RT). 12 weeks old, C57BL/6 male mice received 3 i.v. injections of 4 mg/Kg Dox each, reaching a cumulative dose of 12mg/kg of Dox. Data was obtained at 2, 6 and 15 weeks after last injection. RT-treated mice were locally irradiated with 2 and 16Gy localized to the heart, and experiments performed 20 and 50 weeks after irradiation. M-mode Echocardiography showed a significant reduction of the ejection fraction (EF) at 15w after last injection of Dox. Consistently, the amplitude of $[Ca^{2+}]$i transients was decreased (F/F0: 4.10 ± 0.25, n=11 control cells, vs 3.22 ± 0.14, n=15 Dox, $p<0.01$) and slowed (Tau in ms: 138.98 ± 6.17, n=11 control vs 169.63 ± 11.91, n=15 Dox, $p<0.05$) at 15w with a significant increase in the frequency of Ca^{2+} sparks, but without changes on the SR Ca^{2+} content. RT induced a LV enlargement associated with a significant reduction of EF in low and high dose irradiated mice group at both time lapses. However, Ca^{2+} handling was minimally affected by RT. After 50w of 16Gy RT, we observed a faster decay of $[Ca^{2+}]$i transients with unaltered amplitude and SR Ca^{2+} content. Our results indicate that the depressed cardiac function after Dox treatment involves $[Ca^{2+}]$i transient reduction, while in RT-induced cardiotoxicity the Ca^{2+} alteration may not be involved likewise.

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Mechanisms of Anthracycline-Induced Dysfunction of Calcium Handling Proteins in the Heart

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Anthracyclines are powerful chemotherapy agents, whose use is limited due to the onset of potentially fatal cardiotoxicity which includes arrhythmogenesis and heart failure. Several proteins important in intracellular Ca^{2+} signalling have been identified as drug binding targets, including the cardiac ryanodine receptor Ca^{2+} release channel (RyR2), the Ca^{2+} binding protein calsequestrin (CSQ2) and the Sarco/Endoplasmic Reticulum Ca-ATPase (SERCA2a). The effects of the drug metabolites have been poorly characterized but are believed to be important in the devastating cardiac effects of these drugs.

The functional effect of doxorubicin and its metabolite, doxorubicinol on RyR2 was assessed by adding clinically relevant drug concentrations to single RyR2 channels in lipid bilayers. Both drugs caused biphasic modulation of RyR2 activity where there was an early increase in channel activity followed by a later, inhibitory phase. RyR2 channel activation, but not inhibition, could be reversed by drug washout, typical of a ligand binding effect. Conversely, the irreversible nature of the inhibitory effect suggested a non-ligand binding effect. Treatment with doxorubicin/doxorubicinol reduced the number of thiols on RyR2, indicative of drug-induced thiol oxidation. Additionally, doxorubicinol abolished the response of RyR2 to changes in luminal Ca^{2+}. Further experiments revealed that the loss of luminal Ca^{2+} sensing was due to an interaction between doxOL and CSQ2. Finally, we found that doxorubicinol inhibits SERCA2a Ca^{2+} uptake into SR vesicles and that this was prevented by pretreatment with DTT. These results provide novel insight into the cellular mechanisms of anthracyclines. We suggest that by targeting multiple Ca^{2+} handling proteins in cardiac muscle, anthracyclines severely disturb cardiomyocyte Ca^{2+} homeostasis and that these effects have an important role in the onset of anthracycline-mediated arrhythmia and heart failure.

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Predictive Drug Toxicity Profiling using a New Mode of Calcium Signal Analysis in Human Es-Derived Cardiomyocytes

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Contemporary drug screening platforms often fail to resolve hazards related to chronic toxicity. Many drugs induce a defective homeostatic state, which may acutely appear 'normal', but the chronicity of which provokes phenotypic dysfunction (latent toxicity). We showed that abnormal Ca^{2+} signals can be embedded within perceptibly normal steady-state Ca^{2+} cycling. In order to expose these hidden Ca^{2+} patterns, we developed methods to decrypt intra- and inter-cellular Ca^{2+} signals in individual cardiomycytes and in multicellular populations. In this proof-of-concept study, we used confocal imaging and pattern analysis of Ca^{2+} handling in spontaneously contractile Cytiva