including arrhythmia and heart failure. Acute doxorubicin cardiotoxicity is multifaceted and includes the formation of reactive oxygen species, apoptosis, and disturbances in calcium handling within the intracellular sarcoplasmic reticulum (SR) store. Our focus is on the protein targets within the SR that bind doxorubicin, namely the ryanodine receptor (RyR2) calcium release channel and the calcium binding protein, calsequestrin (CSQ2) which also regulates RyR2, and how drug binding disturbs SR calcium homeostasis. Such disturbances are known to cause cardiac dysfunction. Our previous *in vitro* assays showed that doxorubicin reduced CSQ2's calcium binding capacity, altered SR calcium homeostasis and impaired RyR2 activity, as a consequence of the drug binding directly to these proteins and to drug-induced RyR2 oxidation.
To investigate the *in vivo* modifications of RyR2 and other SR proteins by doxorubicin, we developed an acute mouse model of doxorubicin cardiotoxicity. Doxorubicin treatment reduced expression of RyR2, the SR calcium pump SERCA and CSQ2 and lowered the fraction of CSQ2 associated with RyR2. The decline in SERCA and RyR2 expression and the reduced proportion of RyR2 that can be activated by CSQ2 may underlie the significantly perturbed SR calcium release after doxorubicin treatment. In addition, doxorubicin treatment caused "hyper" phosphorylation of RyR2 at S2808 and significant dissociation of the RyR2 dephosphorylating enzymes PP1 and PP2A. Hyperphosphorylation of S2808 is known to cause RyR2 dysfunction, afterdepolarizations, arrhythmia and heart failure. It is likely other modifications in RyR2 function (oxidation, ligand-binding) in addition to RyR2 hyperphosphorylation and reduced CSQ association underlie the multi-faceted disturbances in calcium release that contribute to the clinical cardiotoxicity induced by doxorubicin treatment.

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Ros and CaMKII Modulate Sodium Overload-Induced Calcium Leak from Ryanodine Receptors in Ventricular Myocytes
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Introduction: Elevation of the diastolic free calcium concentration ($[Ca^{2+}]_{cyt}$) alters excitation-contraction coupling and contractility in cardiac myocytes. It is known that Na^+ overload increases the levels of diastolic $[Ca^{2+}]_{cyt}$ and induces arrhythmias via reverse mode of Na^+/Ca^{2+} exchanger. We hypothesized that Ca^{2+} leak induced by oxidation/phosphorylation of ryanodine receptors (RyRs) contributes to the rise in $[Ca^{2+}]_{cyt}$ during Na^+ overload.
Methods: We used confocal fluorescence imaging of intact and membrane-permeabilized isolated rabbit and rat ventricular myocytes to study Ca^{2+} transients, cytosolic sodium ($[Na^+]_{cyt}$) and intracellular reactive oxygen species (ROS) levels. Cellular Na^+ overload was induced using 5 nM anemone toxin-II (ATX-II), a late Na^+ current (I_{NaL}) enhancer.
Results: ATX-II-induced $[Na^+]_{cyt}$ overload caused abnormal Ca^{2+} transients in intact rabbit and rat myocytes. Additionally, high cytosolic Na^+ increased ROS levels and activated CaMKII. These effects were abolished by the INa inhibition with ranolazine and TTX, CaMKII inhibition with KN93 and AIP or by the application of antioxidants such as DTT and coenzyme Q10. In the experiments with membrane-permeabilized myocytes, rapid elevation of $[Na^+]_{cyt}$ from 5 mM to 20 mM resulted in an increase of ROS production in mitochondria and Ca^{2+} leak from RyRs by measuring the frequency of spontaneous Ca^{2+} waves. The CaMKII inhibitors, KN-93 and AIP, attenuated the effects of high $[Na^+]_{cyt}$ on Ca^{2+} signaling.
Conclusions: These data suggest that $[Na^+]_{cyt}$ regulates RyRs activity. An increase in $[Na^+]_{cyt}$ induces ROS production; ROS activates CaMKII leading to altered Ca^{2+} transients and abnormal Ca^{2+} handling.

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Hyperaldosteronism Severely Effects Calcium Handling, Contractility and Action Potentials in Isolated Cardiomyocytes of Rats
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Evidence is increasing that aldosterone is not only regulating renal salt reabsorption but might also be a key player in the development of cardiomyopathies. Especially, chronically elevated levels of aldosterone (hyperaldosteronism) participate in the generation of atrial fibrillation, tachycardia, hypertrophy, fibrosis or inflammation. We elevated blood plasma levels of adult rats by implanting osmotic mini-pumps in 8 week old rats for 8 weeks (1,5 µg/h aldosterone). For the exploration on cellular excitation and contraction we performed isolation of ventricular and left and right atrial myocytes and studied cellular calcium handling, contractility and electrical properties of the cells.
In ventricular myocytes we observed a severe drop-down of resting calcium, 1st amplitude (A1st) and steady-state amplitude (Astst) of global electrically induced calcium transients. For atrial myocytes of the right atrium such changes were rather pronounced while surprisingly, the left atrial myocytes displayed such effects to a much lesser degree. In line with changes in calcium handling contractility of the myocytes was also impaired. Here, right atrial myocytes displayed a pronounced negative contractility-frequency relationship (0.2 - 2 Hz) and their resting/diastolic sarcomer length was massively increased when compared to cells from control animals. When patch clamping the atrial myocytes we also found substantial changes in their action potentials (AP). Interestingly, for both left and right atrial cells, early phases of repolarisation were massively shortened (>50% change). Right atrial cells also showed a reduced AP rise time and their capacitance were significantly reduced (>30%).
Here we demonstrate for the first time a differential effect of hyperaldosteronism on ventricular myocytes and cells from the left and right atrium.
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Regulatory Role of Histidine Rich Calcium Binding Protein (HRC) in Cardiac Sarcoplasmic Reticulum Ca2+ Release Complex
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Ca2+ release from sarcoplasmic reticulum (SR) in cardiac muscle is mediated by a multiprotein complex that includes the RyR, triadin, calsequestrin (CSQ), junctin and HRC. The interaction of these proteins with each other, the luminal SR Ca2+ concentration and the influx of Ca2+ from the L-type Ca2+ channel (LTCC) determines the activation of RyR. Triadin has a charged luminal region which interacts with Ca2+ binding proteins such as CSQ, HRC and thus can directly or indirectly regulate RyR activity. In the present study using an in vitro binding assay and deletion mutants, we found that only the distal region of triadin interacts with RyR, CSQ and HRC and that the 4th KEKE motif (amino acid 202-231) of triadin is the minimal interacting region to interact with all the three proteins. We compared the interaction of triadin with the individual proteins by co-immuno precipitation of triadin interacting partners at different levels of free Ca2+ concentrations. The result showed maximal RyR interaction with triadin at pCa 3, HRC at pCa 4 and, CSQ at pCa 7 and 3. To study whether these proteins compete with each other to interact with triadin we conducted a competitive binding assay. The results showed no competition between RyR (loop II) and CSQ, while a significant competition was noticed between HRC and RyR (loop II) to bind to triadin. Taken together, we suggest that HRC interacts with triadin at intermediate luminal Ca2+ concentration and has an important role in maintaining the refractoriness of Ca2+ release by decreasing RyR activity. (Supported by Korea MEST NRF grant (2011-0002144), the 2011 GIST Systems Biology Infrastructure Establishment Grant and KISTI-KREONET).

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Severe Cardiac Hypertrophy and Depressed Contractility in Transgenic Mice with Co-Overexpression of Calsequestrin and SERCA2a
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Cardiac-specific overexpression of calsequestrin (CSQ) is associated with hypertrophy, depressed contractility, impaired SR Ca^{2+} release and signs of heart failure. The Ca^{2+}ATPase (SERCA2a) represents the major determinant of myocardial contractility. Its stable or transient expression results in increased contractile parameters and higher Ca^{2+} transients. Hence, we tested whether the additional expression of SERCA2a in CSQ-overexpressing hearts results in beneficial functional effects. For this purpose, we generated double transgenic mice overexpressing both CSQ and SERCA2a (TG^{CxS}) by cross-breeding of single transgenic mouse lines (TG^{CSQ} and TG^{SER}). SERCA2a protein expression was increased by 1.4-fold in both TG^{SER} and TG^{CxS} compared to wild-type (WT) hearts. Consistently, at submaximal free Ca^{2+} levels (30 nmoL/L), $^{45}Ca^{2+}$ uptake reached 12%, 25%, 14% and 30% of maximal Ca^{2+} levels in WT, TG^{SER}, TG^{CSQ} and TG^{CxS}, respectively (n=5, $P<0.05$). CSQ was overexpressed by 10-fold in both TG^{CSQ} and TG^{CxS} compared to WT. The expression of triadin was decreased by 53% in both TG^{CSQ} and TG^{CxS} compared to WT ($P<0.05$). The ryanodine receptor expression was reduced by 44%, 33% and 86% in TG^{CSQ}, TG^{SER} and TG^{CxS}, respectively, compared to WT ($P<0.05$). In addition, co-overexpression of CSQ and SERCA2a was