Ryanodine Receptors Type II

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Relocation of Calmodulin in Hypertrophic Neonatal Cardiomyocytes

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An increasing body of evidence suggests that calmodulin (CaM) signaling is involved in the regulation of gene expression in cardiac hypertrophy (HT). According to recent report (Yamaguchi *et al*, 2007) mice expressing RyR2 mutant, that cannot bind CaM, develops cardiac HT. In an attempt to gain further insight into the role of RyR2-bound CaM in the process of development of HT, we have induced HT in neonatal rat cardiomyocytes by endothelin-1 (ET-1), and found distinct differences between the hypertrophied cells and control cells in several aspects. (1) The immuno-stain with anti-CaM antibody is chiefly localized in the cytoplasm of the control cells; whereas, in the HT cells, the stain is accumulated chiefly in the nucleus. (2) The cross linking/Western blot analysis shows that CaM is cross-linked with the RyR2 in control cells, but not in the HT cells. (3) We have prepared fluorescently labeled CaM by conjugating C^{34} mutant of CaM with Alexa 546, and introduced it into the myocytes using Bioporter. The exogenously introduced CaM-alexa is localized mainly in the cytoplasmic region in control cells, but within the nucleus in the HT cells. These findings suggest that stimulation of the neonatal cardiomyocytes with ET-1 dissociates RyR2-bound CaM from the receptor, then the dissociated CaM translocates to the nucleus to activate the HT gene program. In support of this conclusion, dantrolene (a specific blocker of RyR Ca^{2+} channel) inhibited HT in the ET-1-treated cells; at the same time prevented CaM translocation to the nucleus. Furthermore, an antibody raised against the CaM binding domain of RyR2, which was introduced into the cell via Bioporter, also prevented HT in the ET-1-treated cells. (Supported by HL072841)

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Endurance Training Abolish Arrhythmogenic Ca2+ Leak In Cardiomyocytes From Mice Over-expressing CaMKII

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Background: Transgenic (TG) over-expression of calcium/calmodulin-dependent protein kinase IIδC (CaMKIIδC) causes depressed cardiac function, altered Ca2+ handling and increased diastolic sarcoplamic reticulum (SR) Ca2+ leak. The latter may trigger ventricular arrhythmias in heart failure. Aim of the study was to investigate the effects of endurance training on Ca2+ handling and diastolic SR Ca2+ leak in TG mice with over-expression of CaMKIIδC.

Methods: Four CaMKIIδC TG mice underwent high intensity endurance training 5 days per week over 12 weeks and were compared to TG sedentary and wild type (WT). Ca2+ handling, diastolic SR Ca2+ leak, t-tubule density and SR Ca2+ release synchronicity were measured in single cardiomyocytes.

Results: TG mice had depressed cardiomyocyte shortening ($3.3 \pm 1.8\%$ in TG vs. $6.2 \pm 1.2\%$ in WT, $P<0.01$). Ca2+ transient amplitude were lower (Fura-2AM ratio in TG was 0.09 ± 0.03 vs. 0.18 ± 0.02 in WT, $P<0.01$). SR Ca2+ leak over the RyR was significantly larger in TG mice ($19 \pm 3\%$ of total SR Ca2+ vs. $5 \pm 2\%$ in WT, $P<0.01$). Endurance training restored cardiomyocyte shortening ($5.9 \pm 1.3\%$ in TG trained) to WT level. Ca2+ amplitude was also significantly increased (Fura-2AM ratio 0.15 ± 0.02 in TG trained). Endurance training reduced diastolic SR Ca2+ leak to WT levels ($4 \pm 2\%$, $P<0.01$ vs. TG). CaMKIIδC inhibition and not PKA inhibition normalized SR Ca2+ leak in TG and was comparable to TG endurance trained and WT. TG had both reduced t-tubule density ($13 \pm 4\%$ in TG vs. $18 \pm 2\%$ in WT, $P<0.05$) and reduced SR Ca2+ release synchronicity ($P<0.05$), whereas t-tubule density and SR Ca2+ release synchronicity in endurance trained TG were comparable to WT.

Conclusion: Endurance training improved cardiomyocyte function and Ca2+ handling in mice with TG over-expression of CaMKIIδC. Increased diastolic SR Ca2+ leak, t-tubule density and SR Ca2+ release synchronicity was normalized after endurance training.

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Ca^{2+}-calmodulin Increases RyR2 Open Probability Yet Reduces Ryanoid Association With RyR2

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We have previously demonstrated that low, physiological concentrations of Ca^{2+}-calmodulin (Ca^{2+}CaM; 50–100 nM) activate RyR2 channels incorporated into bilayers (Sigalas & Sitsapesan (2007) *Biophys.J.*, 88a). In contrast, we find that Ca^{2+}CaM dose-dependently inhibits [^{3}H]ryanodine binding to cardiac HSR. Since it appears that the [^{3}H]ryanodine binding assay is not accurately reporting the effects of Ca^{2+}CaM on RyR2 Po we have investigated, using the reversible ryanoid, ryanodol, whether Ca^{2+}CaM can directly influence the binding of ryanoid compounds to RyR2 independently of Po. Purified single sheep RyR2 channels were incorporated into artificial membranes under voltage-clamp conditions. In symmetrical solutions containing 250 mM K+, 100 μM free Ca^{2+}, pH 7.2, and 20 mM caffeine to clamp Po to high levels, 1 μM CaM had no significant effect on Po. This agrees with our previous work on native RyR2 channels demonstrating that high levels of CaM can reverse the activating effects of low [Ca^{2+}CaM] but do not significantly lower Po below control values. We find that Ca^{2+}CaM significantly reduces the rate of association of ryanodol to RyR2 but does not affect the rate of dissociation. Ca^{2+}CaM also significantly alters channel behaviour within the ryanodol-modified conductance state. Our results lead to two important conclusions: 1. A Ca^{2+}CaM-induced decrease in the rate of association of ryanoids to RyR2 is consistent with the reduction in [^{3}H]ryanodine binding caused by Ca^{2+}CaM, providing an explanation why the functional effects of Ca^{2+}CaM at the single-channel level are not faithfully mirrored in the [^{3}H]ryanodine binding studies. 2. The important, wider implications of this study are that [^{3}H]ryanodine binding assays, in the absence of other supporting data, may not provide reliable information about the Po of RyR channel populations.

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Phosphorylation Activates RyR2 By Uncoupling The Channel From The Influence Of Cytosolic Ca^{2+} But Also Inhibits RyR2 By A Distinct Mechanism

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Phosphorylation of RyR2 is generally thought to be associated with an increase in open probability (P_o), however, there is disagreement over which phosphorylation sites are responsible. To investigate the gating mechanisms underlying phosphorylation-induced changes in RyR2 P_o, we incorporated native sheep RyR2 into bilayers under voltage-clamp conditions with luminal Ca^{2+} as the permeant ion. Phosphorylation of RyR2 channels above basal levels, either by PKA or by activation of an endogenous kinase (by incubation with Mg^{2+}ATP) leads to massive increases in P_o that are associated with very long open states. The channels become effectively uncoupled from the influence of cytosolic Ca^{2+} because reductions in cytosolic [Ca^{2+}] to sub-activating levels do not reduce open lifetimes nor significantly reduce P_o. Treatment of channels with the phosphatase, PP1 reverses these gating changes returning the channels to a cytosolic Ca^{2+}-sensitive mode of gating. In contrast, to the gating changes described above which are specific for phosphorylation of RyR2 *above* basal levels, complete dephosphorylation of RyR2 by PP1 treatment causes significant increases in P_o. P_o increased from 0.074 ± 0.032 to 0.218 ± 0.028 (SEM;n=4) after incubation with PP1 (5 units). The channel gating associated with completely dephosphorylated channels, or channels phosphorylated to 75% of maximum at serine-2809, demonstrates that the channels are sensitive to cytosolic [Ca^{2+}]. We cannot yet assign specific phosphorylation sites to particular change in channel gating but it is clear that phosphorylation of RyR2 exerts effects which span the entire range of the RyR2 activity landscape. The huge scope for changing RyR2 channel activity suggests that phosphorylation of RyR2 is important for physiological regulation of the channel and that dysregulation of SR Ca^{2+}-release would be expected with prolonged hyperphosphorylation.

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FKBP12.6 Overexpression Blunts Cardiomyocyte Remodeling After Left-ventricular Pressure-overload

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Background. Increased open probability of the ryanodine receptor (RyR) has been implicated in the pathophysiology of heart failure. Since association of FK506 binding protein (FKBP12.6) with RyR enhances its closed state we investigated whether FKBP12.6 overexpression could improve cellular remodeling and Ca^{2+} handling in the setting of pressure-overload. **Methods**. We used a mouse model with heart-specific and conditional overexpression of FKBP12.6. Wild-type (WT) mice and mice overexpressing FKBP12.6 (TG) were submitted to 10 weeks transverse aortic constriction (TAC) and compared