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Tnfα Alters Mitochondrial Function And Ca^{2+} Homeostasis In Ventricular Cardiomyocytes: A Key Role For Caspase-8 Activation

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Tumor necrosis factor α (TNFα), a pro-inflammatory cytokine, is associated with major cardiomyopathy. In the heart, TNFα binding to the TNF receptor 1 (TNFR1) has been implicated in TNFα mediating negative inotropic effects as well as apoptosis. TNFα-TNFR1 activates caspase-8 which leads to caspase-3 activation either directly or following mitochondrial disruption. Here we investigated whether caspase-8-induced mitochondrial dysfunction could lead to TNFα-induced alterations of Ca^{2+} homeostasis. All experiments were performed on freshly isolated rat ventricular cardiomyocytes using multi-photons or confocal microscope. One hour of TNFα application (10 ng/ml) activates caspase-8 as well as caspase-3 measured with carboxyfluroscein-derived specific probes. TNFα depolarized mitochondrial membrane potential (measured with TMRM), and increased mitochondrial superoxyde production (measured with MitoSox). In the mean time, mitochondrial Ca^{2+} decreased, preceding an elevation in resting cytosolic Ca^{2+} fluorescence (Rhod-2 and Fluo-4 measurements respectively) and an increase in spontaneous ryanodine receptors activities (sparks frequency). Alternatively, on field stimulated cells (0.5 Hz), TNFα decreased Ca^{2+} transients' amplitude and SR load. TNFα-mediated alteration in SR Ca2+ function was normalized by antioxidant (NAC; 20 mM). In addition, a broad-spectrum caspase inhibitor (Q-VD-oph; 10 μM) or specific caspase-8 inhibitors (TRP801 and z-IETD-fmk; 10 μM), blocked TNFα effects both on mitochondria and Ca^{2+} handling. On an ischemia-reperfusion model, intra-peritoneal injection of TRP801, 15 min minutes prior reperfusion, prevented long term morpho-functional remodeling. In conclusion, caspase-8 activity appears to mediate TNFα-induced mitochondrial dysfunction which in turn alters global Ca^{2+} handling independently of caspase-3 activation. Caspase-8 inhibition presents a potential therapeutic target.

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Blocking Mitochondrial Ca^{2+} Uptake Increases Matrix Reactive Oxygen Species During Excitation-contraction Coupling In Cardiac Myocytes

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Mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) is taken up by the Ca^{2+}-uniporter (mCU) and stimulates NADH- and ATP-production. Furthermore, the NADH redox state is in equilibrium with the NADPH- and glutathione-pools, and glutathione is required for glutathione peroxidase to eliminate H_2O_2. Thus, we hypothesized that inhibiting mitochondrial Ca^{2+}-uptake could increase H_2O_2 formation. Experiments were performed in guinea-pig cardiac myocytes (n=10-13/group). To monitor $[Ca^{2+}]_m$, myocytes were loaded with rhod-2AM, and then patch-clamped and dialyzed with a pipette solution containing indo-1 to detect cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$). Alternatively, myocytes were loaded with the H_2O_2-sensitive dye CM-DCF, which locates primarily to mitochondria, and then dialyzed with DCF-free pipette solution to remove cytosolic DCF. In these cells, NADH autofluorescence was monitored together with DCF. In voltage-clamp mode, cells were depolarized from -80 to +10mV at 3Hz and exposed to isoproterenol (10/100 nM) for 12 min. Under control conditions, beat-to-beat oscillations of $[Ca^{2+}]_m$ were observed during cytosolic Ca^{2+} transients. Isoproterenol increased the amplitude of both $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ transients and led to diastolic accumulation of $[Ca^{2+}]_m$, but not $[Ca^{2+}]_c$. When $[Ca^{2+}]_c$ transients increased in response to isoproterenol, NADH transiently oxidized, but recovered when diastolic $[Ca^{2+}]_m$ increased. During this transient NADH oxidation, net formation of H_2O_2 increased but returned to baseline levels when diastolic $[Ca^{2+}]_m$ increased and NADH recovered. When inhibiting mitochondrial Ca^{2+}-uptake with the mCU-blocker Ru360 (1μM in pipette solution), diastolic accumulation of $[Ca^{2+}]_m$ was abolished and the recovery of oxidized NADH blunted. Consequently, net formation of H_2O_2 increased compared to control conditions (F/F_0 after 12 min of isoproterenol: 1.7 ± 0.2 vs 1.2 ± 0.1; $p<0.05$). We conclude that mitochondrial Ca^{2+} uptake is required for (a) matching energy supply and demand and (b) keeping the mitochondrial matrix in a reduced redox state to prevent formation of H_2O_2.

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Effects Of Oxysterols On The Sr Ca^{2+} Cycling In Ventricular Myocytes

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Oxysterols are biologically active molecules generated during the oxidation of low density lipoprotein (LDL). Several oxysterols are found in macrophage-derived 'foam cells' from human atherosclerotic tissue. Lipophilic oxysterols penetrate cell membranes and, therefore, can diffuse into the surrounding epithelial, smooth muscle, and cardiac cells from macrophages located in the atherosclerotic plaques or from inflammatory zones. Some cholesterol oxides have been shown to injure vascular endothelial and smooth muscle cells. 7β- and 25-hydroxycholesterol (HC) are the most toxic and the most abundant agents in the group.

We employed confocal microscopy and fluorometry to study the effects of 0.1-10 μM 7β-HC and 25-HC on the mechanisms underlying contraction in rat ventricular myocytes. Our experiments showed that both oxysterols:

(1) inhibit cell responses to electrical stimulations (2 Hz) in a dose-dependent manner;

(2) increase resting cytoplasmic $[Ca^{2+}]$ two-fold (1 Hz stimulation);

(3) slow Ca^{2+} removal from the cytosol in stimulated cells (1 Hz);

(4) reduce the caffeine-induced sarcoplasmic reticulum (SR) Ca^{2+} release by 30-45%;

(5) reduce the appearance of spontaneous Ca^{2+} waves in Ca^{2+}-overloaded intact ventricular myocytes by ~40 % and abolished them in Ca^{2+}-overloaded permeabilized ventricular myocytes;

(6) do not change the frequency of Ca^{2+} sparks in permeabilized ventricular myocytes during 5 minutes after the exposure under normal conditions (100 nM Ca^{2+}) but reduce it by ~40 % in Ca^{2+}-overloaded myocytes (120 nM Ca^{2+});

(7) increased the time constant of the SR Ca^{2+} uptake up to 3 fold in cardiac SR microsomes.

We conclude that oxysterols inhibit SR Ca^{2+} uptake (probably by decreasing the turnover rate of the SR Ca^{2+} ATPase). Our data suggest that the pathological actions of macrophage oxysterols may depend on dysfunctional Ca^{2+} signaling at the cellular and subcellular levels.

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Modulation Of Cardiac Contractility By Antagonism Of Pleckstrin-homology Domain And Akt-1 Silencing

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The pleckstrin-homology (PH) domain is involved in PI3-Kinase-mediated membrane recruitment, and subsequent activation, of signaling pathways, including Akt. PI3-Kinase pathway may modulate beta-adrenergic inotropic effect and Akt disregulation has a central role in diabetic cardiomyopathy. Recent data suggest that Akt may directly modulate sarcoplasmic reticulum (SR) function. Aims: to investigate modulation of cardiac excitation-contraction (EC) coupling by 1) two chemically unrelated compounds with PH-domain affinity (compounds A and B); 2) selective Akt-1 isoform silencing by small RNA-interference (sRNAi). Methods: rat ventricular myocytes were studied at 36.5 °C. Twitch amplitude was measured during field stimulation (2 Hz). Intracellular Ca2+ transients (FLUO 4-AM) was recorded in V-clamped myocytes; SR Ca2+ uptake function was estimated from Ca2+−transient features under inhibition of the Na+/Ca2+ exchanger (by Na+−free conditions). Akt-1 silencing was performed by myocyte transfection with Akt1-specific ds-iRNA oligos, labelled with the fluorescent probe Cy3. Akt phosphorylation levels and activity were tested by western-blot and ELISA. The effect of all interventions was tested in basal conditions and under weak adrenergic activation (isoproterenol 10 nM). Results: Akt-1 phosphorylation and activity were decreased by compounds A and B. Both compounds increased ($p<0.05$) twitch amplitude in basal condition; these effects was enhanced during weak beta-AR stimulation, also the compounds effects are significantly reduced during beta-AR blockade. Akt-1 silencing increased twitch amplitude, enhanced its response to beta-AR stimulation and completely occluded the effect of compounds. The compounds increased SR Ca reuptake rate and EC-coupling gain. Conclusions: 1) chemical antagonism of PH-domain increased contractility by stimulating SR Ca2+ uptake; 2) this effect is likely to result from inhibition of the Akt-1 pathway and involve interaction of the latter with beta-AR-mediated signaling; 3) PH-domain is a novel putative target for inotropical support through enhancement of SR function.

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Peroxynitrite Increases Protein Phosphatase Activity and Promotes the Interaction of Phospholamban with Protein Phosphatase 2a in the Myocardium

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Nitric oxide and superoxide react to form the potent oxidant peroxynitrite. The production of peroxynitrite increases during the pathogenesis of heart failure