4:15

770-2 Experimental Autoimmune Myocarditis is Associated with Enhanced Vascular Intercellular Adhesion Molecule-1 Expression

Jin Kang, Sheri E. Belland, Nitchet Newton, Howard J. Eisen. *Temple University, Philadelphia, PA*

We have previously described a model of cardiac myosin-induced autoimmune myocarditis in Lewis rats. This autoimmune myocarditis is associated with significant myocardial lymphocytic (CD4+) infiltration and left ventricular dysfunction developing 21 days after initial immunization with myosin. Intercellular Adhesion Molecule-1 (ICAM-1) plays an important role in cell recognition processes such as leukocyte activation and leukocyte-endothelial interactions. The role of ICAM-1 in myocarditis is unknown. To determine the relationship of ICAM-1 expression to the development and severity of autoimmune myocarditis, 10 Lewis rats underwent immunization with 1 mg myosin in Complete Freund's Adjuvant on days 1 and 8. As controls, 10 Lewis rats were immunized with Bovine Serum Albumin in Complete Freund's Adjuvant. Control and autoimmune myocarditis rats were sacrificed on days 0, 8, 14, 21 or 28, hearts were removed, frozen, and analyzed by conventional staining with hematoxylin and eosin for histologic grading of the myocarditis and immunohistochemical staining with mouse monoclonal antibodies against rat ICAM-1 and the leukocyte-bound ICAM-1 ligands LFA-α, LFA-β and VLA-4. Specimens were scored in a blinded fashion as 0 (no staining), 1 (mild staining), 2 (moderate staining) or 3 (strong staining) on vascular endothelium and myocardium. Rats immunized with myosin had ICAM-1 expression compared to controls of 1.0 vs. 1.0 on day 8 but 3.0 vs. 1.0 on days 14, 21 and 28. Enhanced expression of LFA-α and LFA-β was noted on days 21 and 28 but not day 14 in rats with myocarditis vs. control rats. No difference in VLA-4 expression was noted in myocarditis rats vs. controls. We conclude that autoimmune myocarditis in rats is associated with significant upregulation of ICAM-1 expression on endothelial cells which pvecedes the development of significant myocardial leukocytic infiltration. LFA-α and LFA-β expression was enhanced at the time of leukocytic infiltration. The upregulation of ICAM-1 may be involved in the pathophysiology of myocarditis.

4:30

770-3 Effects of the Hyperthyroid State on Calcium Transients and Cell Length in Single Rat Ventricular Myocytes

Eric Sweterlitsch, Daniel Williford. *University of Rochester, Rochester, NY*

It has been proposed that the hyperthyroid (HT) state has a direct effect on myocardial contractility by enhancing the function of the sarcoplasmic reticulum (SR) ATP-dependent Ca pump. We tested this hypothesis by simultaneously measuring cytosolic Ca transients and cell length in single ventricular myocytes isolated from HT rats. Rats were made HT by daily injection of 1 mg thyroxin for 18 days and cells were isolated by enzymatic dissociation. Ca transients were measured with fura-2 and cell length by a video edge detector during field stimulation over a range of frequencies (0.3–6 Hz). Experiments were performed at room temperature. HT cells showed a marked decrease in the time to reach peak shortening and 50% and 90% relaxation compared to cells from euthyroid (ET) rats. This effect on cell length corresponded to identical changes in the Ca transients. With higher stimulation frequencies, ET cells displayed increasing diastolic Ca levels as well as larger Ca transients with systole consistent with enhanced Ca loading of the SR. The HT cells did not have an appreciable change in diastolic or systolic Ca levels with higher stimulation frequencies. Additionally, the HT cells remained viable and without evidence of Ca overload at significantly higher frequencies than ET cells. Thus, although the HT cells did not show the initial enhanced contractility of the ET cells in response to higher stimulation frequencies, they were much more resistant to Ca overload which eventually caused functional deterioration in the ET cells at the highest range of stimulation frequencies. This finding, in addition to the marked increase in the rates of diastolic relaxation and Ca sequestration in the HT cells can be explained by enhanced activity of the SR-bound Ca pump. A model for the effect of the HT state on ventricular function mediated via the SR pump is proposed.

4:45

770-4 Single Channel Characteristics of Sarcoplasmic Reticulum Calcium Release Channels from Normal and Failed Human Myocardium

S. Titov, D. Cefali, L.R. Jones, R. Kovacs. *Department of Medical Research, Methodist Hospital, and Krannert Institute of Cardiology, Indianapolis, IN*

Ryanodine receptors or sarcoplasmic reticulum (SR) Ca-release channels play a key role in normal cardiac muscle excitation-contraction coupling. The single channel behavior of the Ca++-release channel in failing human hearts has not been investigated. We compared characteristics of normal and heart failure cardiac SR calcium channels, using the planar lipid bilayer technique. Membrane vesicles were prepared from freshly explanted human hearts either prior to transplantation, or from organ donors with normal hearts not accepted for transplantation (because of the age of the donor). 1.0 μg/ml of membrane protein was used in asymmetric bath solutions: CIS (125 mM TRIS; 250 mM HEPES; 2 uM free calcium) TRANS (250 mM HEPES; 50 mM BaCl2). Lipid bilayers were formed with 5:3 ratio of phosphatidylethanolamine and phosphatidylserine. Data were digitized and analyzed with PCLAMP6 software. Normal heart SR Ca release channels exhibited a slope conductance of 153 pS over the range of voltages from 0 to −60 mV. SR Ca release channels from failing hearts exhibited multiple conductance states, the main state had a slope conductance of 500 pS, and a subconductance of 300 pS over the same voltage range. Normal heart open probability range was 0.05–01. In heart failure open state probability was increased, ranging from 0.1–0.5. Single channel properties of SR Ca release channels from the failing hearts are significantly different than normal hearts. This work is the first attempt to investigate the possible role of SR Ca channel derangements in human heart failure.

5:00

770-5 Chamber Specific Regulation of the Sarcoplasmic Reticulum Calcium ATPase Pump in Human Heart Failure

Loewe O. Go [1], David J. Harnick [1], Steven O. Marx [1], Billie S. Fyfe [1], James Watras [2], Andrew R. Marks [1] [1] *Mount Sinai School of Medicine, New York, NY;* [2] *Univ of Connecticut, Farmington, CT*

Alterations in the expression of Ca^{2+} channels have been described in failing human left ventricle, including down regulation of the ryanodine receptor (RyR)/Ca^{2+} release channel and the sarcoplasmic reticulum Ca^{2+} ATPase pump (SERCA) which are involved in excitation-contraction coupling and relaxation (Cir Res 71:18, 1992). We previously reported chamber specific regulation of the RyR during end-stage human heart failure (Clin Res 42(2):166A, 1994). We investigated whether SERCA is also regulated in the other cardiac chambers during human heart failure. Total RNA and protein homogenates were isolated from the left and right atria (LA, RA) and left and right ventricles (LV, RV) obtained prospectively from 32 cardiac transplant patients and 4 normal controls. Messenger RNA (mRNA) levels of SERCA were quantified using Northern and slot blot hybridizations with a 1.6 kb rat cardiac SERCA cDNA probe and normalized to 28S ribosomal levels. Protein levels of SERCA were quantified using enzyme-linked immunosorbent assays with monoclonal antibodies directed against dog cardiac SERCA. Northern analyses detected a single ≈4 kb mRNA in all regions. Compared to controls, SERCA mRNA expression in failing hearts was decreased in LV by 39% ($p < 0.005$), unchanged in RV, and increased in LA by 255% ($p < 0.005$) and in RA by 338% ($p < 0.025$). Consistent with the mRNA data, immunodetectable levels of SERCA were also reduced in LV by 30% ($p < 0.05$) and unchanged in RV; however, protein levels appeared unchanged or reduced in both atria in contrast to the mRNA. This is the first study reporting simultaneous measurements of SERCA mRNA and protein levels in the human heart. We conclude that chamber specific regulation of SERCA mRNA occurs during end-stage heart failure, corroborated by protein expression in the ventricles. Down regulations of SERCA may contribute to impaired relaxation and increased diastolic tone during heart failure.

5:15

770-6 Effect of Ischemic Preconditioning and Lidocaine Pretreatment on $[Ca^{2+}]_i$ in Rat Hearts

Thomas Stefenelli, Shao T. Wu, Christian P. Zaugg, Geoff Smith, Joan Wikman-Coffelt, William W. Parmley *University of California, San Francisco, California*

Recent data suggest that preconditioning attenuates the increase in free intracellular calcium ($[Ca^{2+}]_i$), $[Na^+]_i$, and $[H^+]_i$ during ischemia by reducing the stimulation of Na^+-H^+ and Na^+-Ca^{2+} exchange. We evaluated the effects of 3 episodes of 5 minutes global ischemia followed by 5 minutes recovery on $[Ca^{2+}]_i$ during control perfusion (Co) and during suppression of the sodium influx (5×10^{-6} M lidocaine; L) with surface fluorometry in Indo-1/AM-loaded isolated perfused rat hearts. During the first ischemia, systolic and enddiastolic $[Ca^{2+}]_i$ were 130% higher in Co as compared to L. The lower $[Ca^{2+}]_i$ concentration in L-treated hearts resulted in better recovery of contractile function during recovery (left ventricular developed pressure Co vs L: $p = 0.01$). During repeated ischemia-recovery cycles peak $[Ca^{2+}]_i$ decreased significantly in Co and remained stable in L (Fig.1). mean % ± SEM; ⋆: $p = 0.05$ vs systolic $[Ca^{2+}]i$ during Co

TUESDAY PM