Comparison of anoxic tolerance of isolated cardiac myocytes from male and female rats

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There is growing recognition that important cardiac-specific sex differences can impact on cardiac function, outcomes of cardiovascular disease, and the cardiac response to stresses such as ischemia. We have recently shown that intact *ex vivo* perfused hearts of male rats are more susceptible to ischemia/reperfusion (I/R) injury than those of female rats (Bell *et al.*, 2008). Ca^{2+} overload during early reperfusion has been shown to play a critical role in cardiac I/R injury and recent evidence has highlighted Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activation as a key factor in cellular apoptosis and necrosis in I/R injury. Whether sex-related differences in myocardial Ca^{2+} handling and/or CaMKII activation underlie the different vulnerability of male and female hearts to ischemic damage has, however, not been directly investigated. The goal of this study was to examine whether the enhanced susceptibility to I/R injury of male hearts could be attributable to differences in cardiac myocyte Ca^{2+} handling and/or CaMKII activity.

Hearts from 12-14 week old male and female Sprague Dawley rats were perfused in Langendorff mode with 50 mg/ml collagenase to enzymatically disperse the cardiac myocytes. The freshly isolated cells were loaded with the Ca^{2+} indicator fura-2 and placed in a superfusion chamber on the stage of an inverted fluorescence microscope. Fura-2 fluorescence (ratio 380/365 nm) and cell shortening (edge detection) were monitored using an Ionoptix system (Ionoptix Corporation, Maryland, USA). Cells were paced at 4Hz and subjected to a protocol designed to mimic ischemia and reperfusion. Following establishment of a steady-state in control solution the cells were superfused with a 'simulated ischemia' solution for 20 min followed by 'reperfusion' with control solution for 30 min. The composition of the control solution was (mM) NaCl (146), KCl (4.7), NaH_2PO_4 (0.35), $MgSO_4$ (1.05), $CaCl_2$ (2), glucose (11), HEPES (10), pH 7.4; while the 'simulated ischemia' solution contained (mM) NaCl (136), KCl (8), NaH_2PO_4 (0.35), $MgSO_4$ (1.05), $CaCl_2$ (2), Na-lactate (10), HEPES (10), pH 6.8. The 'simulated ischemia' solution was equilibrated with 100% N_2 and during the period of simulated ischemia the superfusion chamber was also maintained in a 100% N_2 environment. Cells were studied in either the absence or presence of the CaMKII inhibitor KN-93. For the KN-93 experiments cells were pre-treated with KN-93 for at least 15 min prior to use and KN-93 was present (5 μM) in all solutions used throughout the recording period.

Under control conditions the baseline amplitude of the Ca^{2+} transient and cell shortening were significantly lower in the female myocytes compared to the male myocytes. During simulated ischemia cell shortening initially decreased substantially, together with some diastolic cell lengthening, but then recovered somewhat with continued exposure to the 'simulated ischemia' solution. Upon 'reperfusion' with control solution cells exhibited diastolic contracture and variable recovery of contractile function. There was a marked difference in cell mortality during 'reperfusion' with only a 33% survival rate in male cells as compared to nearly 90% survival in female cells. Among the surviving cells no significant differences were detected in contractile recovery or Ca^{2+} handling between male and female myocytes during the I/R protocol. CaMKII inhibition attenuated diastolic contracture during 'reperfusion' in both male and female myocytes but did not significantly improve contractile recovery. CaMKII inhibition was, however, associated with markedly improved preservation of cell viability during 'reperfusion'.

These results suggest that male vulnerability to I/R injury may be due to an enhanced susceptibility of male myocytes to I/R-induced necrosis rather than depressed contractile recovery of surviving cells. Enhanced CaMKII activity in male cardiac myocytes, possibly due to higher steady-state cell Ca^{2+} levels, may contribute to their greater vulnerability to I/R damage compared to female cardiac myocytes.

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