An augmented CaMKII response in the post-ischemic female heart is not proarrhythmic – towards understanding the sex-specificity of CaMKII pathophysiology

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Marked differences in male/female cardiomyocyte Ca^{2+}-handling and ischemic vulnerability have been identified, though information is lacking about the basic mechanisms responsible for these sex differences. Experimentally, female cardiomyocytes have been shown to operate at a relatively lower Ca^{2+} cycling load (*vs* males), which may be significant as Ca^{2+} is a major causative factor in many of the pathologies associated with ischemia/reperfusion (arrhythmogenesis, contractile dysfunction, cardiomyocyte death). Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a key regulator of myocardial Ca^{2+}-handling proteins, and is shown to mediate Ca^{2+}-related pathologies in ischemia/reperfusion, including reperfusion arrhythmias. However, this work has almost exclusively been performed in males and the sex-specificity of CaMKII actions remains undefined. The aim of this study was therefore to determine the actions of CaMKII in the female heart under normoxic, post-ischemic and Ca^{2+} overload conditions, and assess how this relates to the pro-arrhythmic role of CaMKII in ischemia/reperfusion.

Isolated hearts from male/female Sprague Dawley rats were Langendorff-perfused (2.0mM Ca^{2+}, 37°C, non-paced) and treated with a CaMKII inhibitor (KN93, 0.5μM) 10min immediately before/after global ischemia (25min; n=8, 12-16wk). Left ventricular function was measured with an intraventricular balloon and arrhythmogenic incidence assessed. A further set of hearts were subjected to 25min ischemia and snap frozen at 2min reperfusion (*vs* time-matched aerobic control, n=8-10) to assess expression/phosphorylation status of CaMKII and associated proteins by SDS-PAGE/Western blotting. Finally, to establish the contribution of acute Ca^{2+} overload on this CaMKII response, additional hearts were challenged with high Ca^{2+} (4mM) for 2min, and similar molecular analyses performed.

Under basal, normoxic conditions, female hearts exhibited lower CaMKII autophosphorylation (autoP-CaMKII; arb. units, male *vs* female; 0.76±0.10 *vs* 0.49±0.06, $P<0.05$), phospholamban phosphorylation (PLB-Thr17, CaMKII-specific; 0.062±0.010 *vs* 0.03±0.008, $P<0.05$), and ryanodine receptor phosphorylation (RyR-Ser2814, CaMKII-specific; 1.4±0.3 *vs* 2.5±0.8, $P<0.05$). Post-ischemia, female hearts exhibited less ventricular tachycardia/fibrillation and were unresponsive to CaMKII inhibition. This contrasted with significant anti-arrhythmic actions of KN93 in males (untreated *vs* KN93, duration s; male 553±11 *vs* 305±72*, female 96±40* *vs* 117±51*; *$P<0.05$ *vs* male).

Surprisingly, this reduced arrhythmic severity in females was associated with an augmented CaMKII response at 2min reperfusion. Indeed, autoP-CaMKII (1.5±0.1 *vs* 2.7±0.3, $P<0.05$), PLB-Thr17 (1.3±0.2 *vs* 2.3±0.4, $P=0.05$), and RyR-Ser2814 (1.4±0.3 *vs* 2.5±0.8, $P<0.05$) were all increased in females *vs* males. In hearts perfused with high Ca^{2+}, autoP-CaMKII was also increased in females *vs* males (0.96±0.06 *vs* 1.22±0.08, $P<0.05$) and the extent of increase in PLB-Thr17 (fold change over basal; 1.5±0.2 *vs* 2.8±0.5, $P<0.05$) was greater. This increased CaMKII response to high Ca^{2+} in female hearts was associated with an enhanced capacity to maintain stable end diastolic pressure, whereas this parameter was found to be elevated in males.

These data indicate that upregulation of CaMKII activity is accentuated in females, even though arrhythmias are reduced in early reperfusion. This suggests that the relationship between CaMKII activation and arrhythmogenesis in females is distinctive, and that the actions of CaMKII are more complex than previously described.