Augmented CaMKII recruitment despite less reperfusion arrhythmias in female hearts – a matter of differential post-translational modification of CaMKII splice variants?

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Ischemic heart disease is the leading cause of death in men and women in Australia. Lethal arrhythmias account for a high proportion of deaths from ischemic heart disease, yet the underlying mechanisms remain poorly understood. Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a key regulator of myocardial Ca^{2+}-handling proteins, which mediates Ca^{2+}-related pathologies including cardiac hypertrophy, heart failure, and reperfusion arrhythmias (Bell *et. al.*, 2012). Expressed as two splice variants (δ_B and δ_C) with postulated, but not well-defined selective targets, CaMKII activity can also be maintained through autophosphorylation or oxidation (P-CaMKII & ox-CaMKII). Little is known about whether these splice variants are differentially susceptible to autophosphorylation/oxidation, and how this may influence sex-specific arrhythmogenesis. The aim of this study was to assess the role of CaMKII in reperfusion arrhythmias in male and female hearts, and identify the post-translational molecular processes involved.

Isolated hearts from male/female Sprague Dawley rats were Langendorff-perfused (2.0 mM Ca^{2+}, 37°C, non-paced, $n = 8$) and subjected to one of four pathological perfusion protocols; (i) 20 mins global ischemia (37 C) and 10 mins reperfusion, (iii) 20 mins global ischemia and 2 mins reperfusion, (iii) hydrogen peroxide (200 μM) for 2 mins, and (iv) high Ca^{2+} (4 mM) perfusate for 2 mins. Ventricles were homogenised and separated into approximate cytosolic, membrane and nuclear/myofilament fractions for subsequent Western blot analysis.

Ventricular tachycardia/fibrillation during the first 10 mins of reperfusion was significantly lower in female hearts *vs* males (517 ± 84 *vs* 59±18 secs, $p < 0.05$), despite an augmented upregulation in females of P-CaMKII (1.5 ± 0.1 *vs* 2.7 ± 0.3 arb units, $p < 0.05$) and phosphorylation of its downstream substrates, phospholamban (PLB-Thr17; 0.9 ± 0.1 *vs* 1.7 ± 0.4 arb units, $p < 0.05$) and ryanodine receptor (RyR-Ser2814; 1.3 ± 0.1 *vs* 2.2 ± 0.2 arb units, $p < 0.05$) *vs* males. This contrasts with the current literature (male) linking CaMKII activation with reperfusion arrhythmias. An in-depth analysis assessing P-/ox-CaMKII and PLB-Thr17 in individual hearts revealed some novel relationships. When oxidation of CaMKII was promoted (H_2O_2), an inverse relationship between PLB-Thr17 and ox-CaMKII was observed, with low ox-CaMKII levels associated with high PLB-Thr17. Conversely, in hearts perfused with high Ca^{2+}, to optimally increase autophsophorylation, P-CaMKII and PLB-Thr17 levels were modulated in parallel, indicating post-translational modification influences CaMKII substrate specificity. Furthermore, analysis of sub-fractionated homogenates showed the CaMKIIδ_B splice variant was predominantly localized in the nuclear/myofilament fraction (80% of total expression), whilst CaMKIIδ_C was enriched in the membrane fraction (66% of total). Interestingly, P-CaMKII and ox-CaMKII closely co-localised with CaMKIIδ_B and CaMKIIδ_C respectively, suggesting a differential susceptibility of splice variants to autophosphorylation and oxidative modifications.

These data challenge the canonical view of CaMKII as a pro-arrhythmic mediator, and suggest its arrhythmogenic actions may be dependent on the specific cardiomyocyte subcellular localisation. In females, arrhythmic vulnerability may be suppressed due to an augmented generation of 'cardioprotective' P-CaMKIIδ_B possibly limiting the deleterious actions associated with ox-CaMKIIδ_C.

Bell JR, Curl CL, Ip WT & Delbridge LM. (2012) *International Journal of Cardiology* **59**, 112-18.