Augmented CaMKIIδ activation enhances stress resilience in Ca^{2+} loaded female aromatase-deficient mouse cardiomyocytes

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The role of sex steroids in cardioprotection is contentious, with large clinical trials investigating hormone supplementation failing to deliver outcomes expected from observational studies. Greater mechanistic understanding is required regarding how sex and sex steroids influence myocardial function and stress responses. Aromatase catalyses the conversion of testosterone to estrogen and has been shown to exert important local actions in some extragonadal tissues (e.g. brain, bone). We have shown aromatase is expressed in the heart, and disrupting aromatase activity (high testosterone, low estrogen) increases cardiac functional recovery and tissue salvation post-ischemia (Bell *et al.*, 2011). Primarily assessing cardiomyocyte contractility under Ca^{2+} stress conditions in aromatase knockout (ArKO) mouse hearts, the aim of this study was to determine the cellular aetiology of aromatase inhibition in promoting post-ischemic cardiac recovery. Hearts were extracted and cardiomyocytes isolated from animals anesthetised by intraperitoneal injection of sodium pentobarbitone in combination with heparin (200mg/kg and 200IU/kg respectively). Isolated cardiomyocytes from female ArKO and ArWT mice (n=12-14) were loaded with the Ca^{2+}-sensitive Fura2-AM fluorescent indicator (2.5μM) and challenged with serial increases in $[Ca^{2+}]_o$ (1, 2, 3mM). Cytosolic Ca^{2+} transients and contractility were measured simultaneously using microfluorimetry and edge-detection methods (IonOptix, MA, USA). Parallel immunoblot studies on isolated hearts subjected to ischemia/reperfusion (n=5) were undertaken to assess both post-translational modifications to Ca^{2+}/calmodulin-dependent protein kinase II delta (CaMKIIδ) and downstream target phosphorylation status. Finally, gene expression profiles (n=3; MouseWG-6 Expression BeadChip, Illumina) were compiled to identify core cellular processes that are influenced by changes in systemic/cardiac aromatase levels in females. Ca^{2+} transient amplitude was increased in ArKO myocytes at higher $[Ca^{2+}]_o$ (ArKO *vs* ArWT, F360:380, 3mM; 0.77±0.05 *vs* 0.48±0.08; $P<0.05$), though no difference in diastolic $[Ca^{2+}]_i$ was detected. Accordingly, twitch amplitude was increased at higher $[Ca^{2+}]_o$ (L/L_0, 3mM; 9.4±0.63 *vs* 5.9±0.38; $P<0.01$). This was associated with an increased rate of rise ($P<0.05$) and decay $P<0.05$) of the Ca^{2+} transient and maximum rate of myocyte shortening ($P<0.01$) and lengthening ($P<0.01$) in ArKO myocytes. In isolated and reperfused hearts, autophosphorylation of CaMKIIδ (Thr287; arbitrary units; 1.18±0.05 *vs* 1.00±0.04; $P<0.05$) and downstream protein phosphorylation of phospholamban (Thr17; arbitrary units; 1.30±0.18 *vs* 0.78±0.09; $P<0.05$) was greater in ArKO. Microarray differential gene expression analysis revealed a significant effect of aromatase ablation on genes related to cardiac Ca^{2+} handling and myofilament structure and function. These findings provide novel evidence that the high testosterone, low estrogen status of the female ArKO modifies the gene expression profile to modulate inotropic support via optimized Ca^{2+}-handling in response to stress, with modest impact on basal function. This suggests therapeutic interventions targeting the inhibition of aromatase may have a role in cardioprotection, particularly in women.

Bell JR, Mellor KM, Wollermann AC, Ip WTK, Reichelt ME, Meachem SJ, Simpson ER, Delbridge LMD. (2011) Aromatase deficiency confers paradoxical postischemic cardioprotection. *Endocrinology* **152**: 4937-4947.