Cardiac ischemia - different contexts and different consequences

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Clinically, significant differences are described in relation to occurrence and outcomes of myocardial infarction in women and men. The presence of the coincident co-morbidities, hypertrophy and diabetes, further influence the discrepant male-female consequences of an ischemic event. The mechanistic bases for these contextual disease differences are not well understood. Experimentally, we have demonstrated that female and male cardiomyocytes have different contractile states (Curl *et al.*, 2001). Female myocytes function at lower Ca^{2+} operating levels, exhibiting lower systolic and diastolic Ca^{2+} levels associated with smaller twitch size than males under comparable basal conditions. Given these underlying sex differences in Ca^{2+} handling and contractile status, it could be anticipated that stress and disease states would have different functional impacts.

We and others have shown that responses to ischemic stress differ in male and female hearts. We have developed an inbred rat strain which we have identified as the 'Normal Heart Rat' (Harrap *et al.*, 2002) and have used this model to characterize adult sex differences in the response to ischemic stress. Using *ex vivo* instrumented heart preparations, we have demonstrated that after ischemia, reperfused female NHR hearts recover contractile function to a greater extent than male NHR, with higher developed pressure and contractility (Bell *et al.*, 2008).

The 'RISK' (Reperfusion Injury Survival Kinase) pathway is thought to be key to achieving post ischemia/reperfusion recovery. Protective kinase signalling includes PI3K activation of Akt and also PKC activation of ERK1/2. Estrogen is a recognized activator of the PI3K /Akt pathway, and in the female myocardium this signalling axis is more prominent. Thus, in the female heart there is additional capacity for estrogen modulation of the response to ischemia through Akt signalling. Consistent with this is our finding that in the female NHR, enhanced reperfusion recovery is associated with elevated Akt and ERK1/2.

Sex differences in response to ischemic stress become more apparent in a context of cardiac hypertrophy. As a disease model to complement the NHR, we have developed the 'Hypertrophic Heart Rat' from the same parental cross origins (Harrap *et al.*, 2002). Both female and male adult HHR exhibit significant left ventricular hypertrophy. We have established that in the female HHR, there is selective suppression of contractile recovery after ischemic stress, accompanied by reduced Akt and ERK levels. Thus the female 'advantage' seen in the NHR is undermined when hypertrophy is also present.

Recently, we have provided the first evidence that cardiac estrogen production may occur (Bell *et al.*, 2011). In wildtype rodents we have demonstrated aromatase expression - indicative of the capacity for local androgen-to-estrogen conversion. To more directly evaluate the role of estrogen in modulating cardiac recovery from ischemia, we have examined the functional responses of hearts of aromatase deficient mice, exhibiting systemic and tissue suppression of estrogen production, with testosterone elevation. Paradoxically, hearts from these female mice show enhanced contractile recovery after an ischemic event, albeit increased arrhythmogenic vulnerability (Bell *et al.*, 2011). These investigations suggest that estrogen cardioprotection is context specific, and that sex steroid balance is an important determinant of ischemic vulnerability.

Finally, there is indication that male and female cardiomyocytes respond in a fundamentally different way to energy stress. Our preliminary experiments suggest that a fasting stress is associated with sex specific signalling activation profiles - findings with implications for energy mobilization in the ischemic myocardium and energy access in the diabetic myocardium. Together these explorations of cardiac stress responses suggest that context is important in determining consequence. Ischemia, hypertrophy and energy status are important interacting factors in regulating male- and female-specific outcomes.

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