Different cardioprotection strategies in ageing

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Pre-menopausal women are less likely to suffer from heart disease than men, but this female advantage disappears with age. This suggests that estrogen may influence the expression of cardiovascular disease. The discovery that individual cardiomyocytes possess receptors for estrogen has fueled interest in the effects of estrogen on the heart. Even so, we know very little about this, in part because most studies in preclinical models still use young, mostly male animals.

Our group has explored cellular mechanisms that regulate intracellular calcium handling and contraction in individual cardiomyocytes isolated from anesthetized young and old rats and mice of both sexes. In younger animals (3-6 months of age), we discovered that calcium transients and contractions are much smaller in ventricular myocytes from female animals when compared to males. These sex differences are not due to differences in calcium current or in the calcium content of the sarcoplasmic reticulum (SR). This lower "gain" of SR calcium release (calcium release per unit calcium current) in myocytes from females arises from smaller subcellular SR calcium events known as calcium sparks (Farrell *et al*., 2010; Parks *et al*., 2014). We have also found that these sex differences are due to changes in the cAMP/protein kinase A (PKA) pathway that regulates SR calcium release. Indeed, basal levels of cAMP are lower in cells from females than males due to increased degradation *via* phosphodiesterase 4B. Lower cAMP levels in females may attenuate PKA phosphorylation of key calcium handling proteins including ryanodine receptors in females (Parks *et al*., 2014). In other experiments, we ovariectomized (OVX) female mice to mimic menopausal changes and reduce estrogen levels. This caused the size of cardiomyocyte calcium transients and contractions to increase dramatically, due in part to an increase in the size of calcium sparks in OVX cells (Fares *et al*., 2012).

These results suggest that estrogen may suppress SR calcium release. Interestingly, SR calcium content also increases dramatically following OVX (Fares *et al*., 2012) and this is similar to the increase we observe in cells from very old (24 months of age) female mice (Howlett, 2010). This increase in SR calcium load leads to an increase in spontaneous SR calcium release in cardiomyocytes from both aged and OVX animals (Fares *et al*., 2012; Ross & Howlett, 2012; Fares *et al*., 2013). These findings demonstrate that low estrogen states such as aging and OVX disrupt the ability of cardiomyocytes to regulate internal calcium levels and lead to SR calcium overload. High levels of SR calcium can have negative consequences for the heart. For example, we found that ventricular myocytes from young adult females are more resistant to ischemia and reperfusion injury than cells from young adult males. Interestingly, age and OVX abolish these beneficial effects in females and induce cellular calcium dysregulation (Ross & Howlett, 2012).

These findings suggest that beneficial effects of estrogen in ischemia and reperfusion are mediated, in part, by effects on cardiomyocytes. These findings are important, because calcium is required to activate cardiac contraction, but too much or too little can promote cardiovascular disease. Thus, age-dependent changes in estrogen levels may modify calcium handling at the cellular level and contribute to the rise in cardiovascular disease in older women.

Fares E, Parks RJ, MacDonald JK, Egar J, Howlett SE. (2012). *J Mol Cell Cardiol* **52**, 32-42.
Fares E, Pyle WG, Ray G, Rose RA, Denovan-Wright E, Chen RP, Howlett SE. (2013). *PLoS One* **8**, e74719.
Farrell SR, Ross JL, Howlett SE. (2010). *Am J Physiol* Heart Circ Physiol **299**, H36-45.
Howlett SE. (2010). *Am J Physiol* Heart Circ Physiol **298**, H659-70.
Parks RJ, Ray G, Rose RA, Howlett SE. (2014). *J Mol Cell Cardiol* **75**, 162-173.
Ross JL, Howlett SE. (2012). *PLoS One* **7**, e38425.