Age-associated changes in cardiac excitation-contraction coupling in ventricular myocytes isolated from male and female Fischer 344 rats

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Mammalian hearts show an age-related decline in contractile function, in particular when heart rates are rapid or when hearts are stimulated by catecholamines (Lakatta & Levy, 2003). Similar age-related deficits in contractile function also are present in ventricular myocytes isolated from aged hearts (Xiao *et al.,* 1994; Lim *et al.,* 2000). However, most previous studies of excitation-contraction (EC)-coupling in aged myocytes have been conducted in cells from male animals only, or from animals where the sex has not been specified. We recently showed that age-associated changes in EC-coupling are more prominent in myocytes from male mice compared to cells from female mice (Grandy & Howlett, 2006). The goal of this study was to determine whether age-related alterations in EC-coupling were affected by the sex of the animal in the Fischer 344 rat, a commonly used animal model of ageing.

Young adult (approximately 3 months) and aged (approximately 24 months) male and female Fischer 344 rats were anaesthetized with an intra peritoneal injection of sodium pentobarbital (220 mg/kg). Ventricular myocytes were isolated by enzymatic dissociation as described previously (O'Brien & Howlett, 2008). All studies were performed at 37°C. Voltage clamp experiments were conducted with high resistance microelectrodes. Cells were paced with a series of conditioning pulses delivered at a frequency of 2 Hz prior to a test step from -40 to 0 mV. Myocytes were loaded with fura-2 AM to measure intracellular calcium concentrations. Unloaded cell shortening was measured simultaneously with a video edge detector. Sarcoplasmic reticulum (SR) calcium content was assessed by rapid application of 10 mM caffeine.

Results showed that myocyte length increased with age in males, but the amplitude of contraction normalized to cell length (fractional shortening) declined with age in the male group (mean contractions were 6.7 ± 0.6 *vs* 2.4 ± 0.4 % for young adult and aged males; n = 19-23 cells/group). Calcium current density also declined with age in cells from males. Calcium transient amplitudes and rates of rise were significantly smaller in aged male myocytes compared to young adult cells (mean calcium transient amplitudes were 47.7 ± 4.6 *vs* 28.1 ± 2.1 nM for young adult and aged males; $p < 0.05$). The SR calcium content did not change with age in male myocytes, but the rate of calcium released per unit calcium current density (an estimate of EC-coupling gain) declined with age in male myocytes (values were 322.8 ± 54.8 *vs* 186.8 ± 23.7 (nM/s)/(pA/pF) for young adult and aged males; $p < 0.05$). Furthermore, the fractional release of SR calcium was reduced in myocytes from aged males compared to younger cells. These results indicate that there is a marked age-related decline in cardiac contractile function in ventricular myocytes from male rats. Results in myocytes isolated from female rat hearts were markedly different. In contrast to results in male animals, cell length was unaffected by age and the degree of fractional shortening was similar in young adult and aged myocytes from female hearts (mean contractions were 4.9 ± 0.7 *vs* 4.9 ± 0.5 % for young adult and aged females; n = 16/cells/group). Furthermore, calcium transient amplitudes and rates of rise were unaffected by age in female myocytes (mean calcium transient amplitudes were 37.5 ± 5.5 *vs* 30.9 ± 3.7 nM for young adult and aged females). Calcium current density did decline with age in cells from females. Still, SR calcium content actually increased markedly in aged female myocytes compared to cells from younger animals (mean values were 49.0 ± 7.5 *vs* 147.3 ± 28.5 nM for young adult and aged females; $p < 0.05$), while fractional SR calcium release declined. However, the gain of EC coupling was not affected by age in myocytes from female rats (mean values were 158.7 ± 18.9 *vs* 202.4 ± 33.3 (nM/s)/(pA/pF) for young adult and aged females).

Together with results of our earlier work, these findings show that age-associated changes in cardiac EC-coupling are profoundly influenced by the sex of the animal. Age-related changes in EC-coupling are more prominent in myocytes from males than in cells from females in two different rodent models. This suggests that there may be a female advantage that limits detrimental effects of age on cardiac EC-coupling. As EC coupling gain was not affected by age in cells from female hearts, the increased SR calcium content might compensate for the decrease in calcium current to maintain contraction.

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