Characterising the measurement of diastolic function in the intact rat cardiomyocyte

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Diastolic heart failure accounts for >50% of all heart failure diagnoses. The pathophysiology of diastolic heart failure is characterized by ventricular stiffness, inadequate filling of the ventricles and elevated ventricular pressure. It is well established that collagen deposition is an important factor in ventricular stiffness. Other evidence suggest that there are also cardiomyocyte specific intrinsic pathologies that underlie ventricular stiffness, although the mechanisms for these are not well understood. The aim of this study was to evaluate intact cell properties and relate these findings directly to the *in vivo* properties of the origin hearts.

Echocardiography was performed in 15 week old male Sprague Dawley rats using a GE Vivid 9 echocardiography system (General Electric, CT, USA). Pulse wave and tissue Doppler were used to determine diastolic function (E/A, MV DecT, E'/A' & E/E'). Cardiomyocytes were isolated by enzymatic digestion and loaded with Ca^{2+} fluorescent dye (Fura-2AM, 5μM). Glass fibres were attached (MyoTak glue) to the longitudinal ends of the cell and paced cardiomyocytes (1Hz, 2.0mM Ca^{2+}, 37^{o}C) were serially stretched (piezo length stepper motor), with sarcomere length/shortening, force development and intracellular Ca^{2+}transients simultaneously measured (Myostretcher, IonOptix, USA).

Comparison of intact heart and single cardiomyocyte diastolic function was undertaken. A correlation between diastolic function measured in the intact heart (MV DecT) and diastolic force measured from the intact cardiomyocyte (r^2=0.9469) was identified, establishing a link between whole heart and single cell diastolic function. To determine if cardiomyocyte function may be altered by loading *per se*, both contractility and intracellular Ca^{2+} were measured in loaded and non-loaded cells – either unattached or attached to a glass fibre (to 'load' the cells). Baseline sarcomere length (unloaded *vs* loaded; μm 1.64±0.03 *vs* 1.63±0.03, N=3, n=10, p=ns), diastolic Ca^{2+} (unloaded *vs* loaded; Ratio (F360/380 nm) 0.54±0.03 *vs* 0.52±0.02, N=3, n=10, p=ns) and systolic Ca^{2+} (unloaded *vs* loaded; Ratio (F360/380 nm) 0.75±0.03 *vs* 0.69±0.03, N=3, n=10, p=ns) were not different in unloaded and loaded cardiomyocytes indicating that attachment of glass fibres does not alter cardiomyocyte morphometry or function. To investigate the relationship between Ca^{2+} and force in 'loaded' cardiomyocytes, cells were stimulated to contract at 1Hz and serially stretched. From this a diastolic force-length relationship was derived. A significant increase in developed force was apparent after stretching (19.3% increase from basal; 11.2% stretch, *P*=0.047). Interestingly this was not accompanied by an increase in intracellular Ca^{2+} with diastolic (0.6% increase from basal), systolic (0% change from basal) and amplitude Ca^{2+} (2.2% decrease from basal) remaining unchanged.

These data indicate that evaluation of cardiomyocyte force development using glass fibre attachment provides a valid measure of cardiomyocyte stiffness without disruption of the dynamic properties of the cell. As *in vivo* heart and *in vitro* single myocyte diastolic function measures correlate, it may be concluded that myocyte origin stiffness represents a quantifiable contribution to intact heart diastolic dysfunction. Further investigation of this relationship in pathophysiological settings is valid.