Testing a methodology for comparative stiffness measurement in isolated loaded intact cardiomyocytes

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Background: Decreased cardiac compliance is a key characteristic of left ventricular diastolic dysfunction which is directly associated with many pathological disease states. Even though cardiomyocytes comprise 70-85% volume of the adult mammalian heart little is known about their contribution to cardiac compliance. The inverse of compliance, cardiomyocyte stiffness, can be determined by stretching cardiomyocytes whilst measuring tension produced. Tension can be mapped against change in cell/sarcomere length to derive a length-tension relationship. The relationship slope can be described as force/Δlength and is a measure of cardiomyocyte stiffness. Tension produced is dependent on the amount of stretch performed on the cardiomyocyte. Therefore, it's crucial that the proportion of cardiomyocyte stretch is reproducible between separate cardiomyocyte preparations. Factors that vary between preparations include the amount of cardiomyocyte subjected to stretch, the intrinsic cardiomyocyte stiffness and diastolic sarcomere length. These factors are hypothesized to alter the amount of electromotive force required by a length-step motor to stretch a cardiomyocyte by a pre-determined figure. Accounting for these factors is crucial for force comparison between separate cardiomyocytes. The aim of this study was to test new methodology for consistent application of cardiomyocyte stretch to ensure reproducible comparison of length-tension relationships between separate isolated cardiomyocyte preparations. To achieve this goal a series of stretch programs were developed to account for the amount of cardiomyocyte subjected to stretch, in addition to stiffness differences between cardiomyocytes.

Methods: Isolated cardiomyocytes were prepared by enzymatic digestion (Collagenase type II, Worthington) and subsequent mechanical dissociation. Isolated cardiomyocytes were attached (MyoTak) at the cardiomyocyte longitudinal surface, and paced cardiomyocytes (2Hz, 2.0mM Ca^{2+}, 37^{o}C) were subjected to two separate stretch protocols (Myostretcher, IonOptix). 1) Four successive 0.5V stretches used to derive the amount of stretch performed by 1V length-step motor electromotive force input (stretch calibration constant μm/V). 2) Six 5% (starting internal fibre diameter) serial stretches were performed using the determined stretch calibration constant and a control constant (0.067μm/V) while simultaneously measuring internal fibre diameter, force development and intracellular Ca^{2+}transients (Fura-2AM, 5μM).

Results: Variation in the stretch calibration group (N=3 animals; n=8 cells) is decreased (30.64 ± 2.42% S.D *vs* 37.4 ± 4.08% S.D) compared to the control constant group (N=3; n=4) indicating that the stretch consistency between cardiomyocytes subjected to individual stretch calibration was higher. Additionally, correlation and slope of linear regression between starting internal fibre distance and %cell stretch were negligible (y=0.0006100x + 30.59, *P*=0.9896, r^2=3.089e-005) in the stretch calibration group compared to control calibration group (y=-0.1359x+48.21, *P*=0.3492, r^2=0.4236). This indicates that any relationship between internal fibre diameter and proportion of cardiomyocyte stretch has been abolished in the stretch calibration group.

Conclusion: Increase in stretch consistency between cells may be crucial to optimising the power of progressive cell stretch coupled with force measurement to derive accurate length-tension relationships and quantify differences in stiffness between cells.