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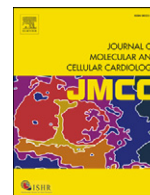

A model of cardiac contraction based on novel measurements of tension development in human cardiomyocytes

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ABSTRACT

Experimental data from human cardiac myocytes at body temperature is crucial for a quantitative understanding of clinically relevant cardiac function and development of whole-organ computational models. However, such experimental data is currently very limited. Specifically, important measurements to characterize changes in tension development in human cardiomyocytes that occur with perturbations in cell length are not available. To address this deficiency, in this study we present an experimental data set collected from skinned human cardiac myocytes, including the passive and viscoelastic properties of isolated myocytes, the steady-state force calcium relationship at different sarcomere lengths, and changes in tension following a rapid increase or decrease in length, and after constant velocity shortening. This data set is, to our knowledge, the first characterization of length and velocity-dependence of tension generation in human skinned cardiac myocytes at body temperature.

We use this data to develop a computational model of contraction and passive viscoelasticity in human myocytes. Our model includes troponin C kinetics, tropomyosin kinetics, a three-state crossbridge model that accounts for the distortion of crossbridges, and the cellular viscoelastic response. Each component is parametrized using our experimental data collected in human cardiomyocytes at body temperature. Furthermore we are able to confirm that properties of length-dependent activation at 37 °C are similar to other species, with a shift in calcium sensitivity and increase in maximum tension. We revise our model of tension generation in the skinned isolated myocyte to replicate reported tension traces generated in intact muscle during isometric tension, to provide a model of human tension generation for multi-scale simulations. This process requires changes to calcium sensitivity, cooperativity, and crossbridge transition rates. We apply this model within multi-scale simulations of biventricular cardiac function and further refine the parametrization within the whole organ context, based on obtaining a healthy ejection fraction. This process reveals that crossbridge cycling rates differ between skinned myocytes and intact myocytes.

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1. Introduction

Computational models of human cardiac function are increasingly used to study cardiac physiology in health and disease [2,3,15,50]. These complex multi-physics and multi-scale cardiac simulations combine models that represent the distinct physiology of the material properties, cellular electrical activity and cellular contraction. When constructing multi-scale models of human function, there are currently many options for characterizing the cellular electrophysiology [19,52] or material properties [48,66]. Detailed biophysical models of cardiac contraction do exist for other species [8,36,56] and have been used to great effect in multi-scale computational models, particularly in the mouse [40,59] and rat [39,51]. These models have the advantage that they can be used to link changes in sub-cellular properties to whole organ contractile performance, predicting key indicators of cardiac function from changes to individual channels or proteins involved in calcium regulation and contractile dynamics. However, there remains a deficit of human biophysical models of tension generation which can be embedded within models of whole organ contraction. Currently whole organ frameworks typically rely on phenomenological models of

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cellular function [30,58] or models preferentially fitted to animal data [64].

A major reason for this disparity between human and animal models is the historically poor availability of contraction data from human isolated myocytes, trabeculae and muscle strips. Specifically, although data for isometric twitch contractile function exists [44,53] and has been used to parametrize contraction models [36,64], comprehensive data sets for human myocyte contractile function which characterize length- and velocity-dependence of tension generation are not available. In addition, many measurements across species are taken at lower temperatures, including measurements of human myofibrils [54], which limits their application for explaining whole organ contractile function. Finally, the assumption that rapid reactions can be approximated as being in steady-state that can be valid in the faster contractile dynamics of rodent hearts may not be valid in the slower contractile dynamics in humans. This limits the ability to simply re-parametrize a small animal model.

In this study we address these deficits by presenting a comprehensive new data set in human cardiac myocytes at 37 °C, measuring the relation between force, calcium concentration and sarcomere length, the response to fast changes in length, and the passive viscoelastic response of these cells. We then use this data to develop a new model of human cardiac contraction, which is suitable for multi-scale modelling, based on the hypothesis that our preparations in skinned cardiac myocytes have crossbridge dynamics consistent with intact cells in whole organ function, and compare our predictions from skinned myocyte data with literature data from intact myocytes and expected whole organ ejection. Finally, we apply this model to quantify differences between skinned myocytes and cellular function in the heart.

2. Methods

Our experimental data includes measurements of passive elasticity, steady-state force calcium relationships at different sarcomere length, and dynamic changes in tension generation during length perturbations in isolated myocytes. Frozen samples of left ventricular myocardium from healthy unused donor hearts were obtained from the Sydney Heart Bank in Australia. The experimental set up and procedures were generally similar to those reported previously [23], except that the force measurements were made at a solution temperature of 37 °C rather than 15 °C.

2.1. Solutions

Relaxing solution contained (mM) BES 100, K propionate 55, Na_2 phosphocreatine 10, Na_2H_2ATP 6.21 (for $MgATP^{2-} = 5$), $MgCl_2$ 6.24 (for $Mg^{2+} = 1$), dithiothreitol 2, EGTA 10, Pi 1.2, AEBSF 1, leupeptin 0.002 and E64 0.002; pH 7.1 at 37 °C, ionic strength 0.20 mol/L. For pre-activating solution 9.85 mM of the EGTA was replaced by HDTA, while for activating solutions 4–10 mM of the EGTA was replaced by CaEGTA. Maxchelator software (http://maxchelator.stanford.edu/) was used to calculate Ca^{2+} concentrations under the different experimental conditions.

2.2. Cell preparation

Fragments of the samples were thawed and single cardiac myocytes and myocyte fragments were prepared by careful glass homogenization of the tissue. The myocytes were permeabilised with 0.5% Triton X-100 v/v (Sigma Aldrich) in relaxing solution for 30 min at room temperature. The homogenate was centrifuged (4000 rpm, 4.5 min) and the myocyte pellet was washed three times in cold relaxing solution (4 °C) to thoroughly remove any traces of Triton X-100. The myocyte suspension was stored on ice and used within 48 h. The ends of a skinned single myocyte were glued at room temperature using Silicon adhesive (Aqua-Sil, Den Braven) or UV-setting glue (OA63 adhesive and Opticure LED-200 UV light source, Norland), between pins extending from a force transducer and a high-speed length controller (403A and 315C-I, Aurora Scientific Inc., Ontario, Canada).

2.3. Measurements of passive viscoelasticity

Passive viscoelasticity was first measured using a series of five 1s step stretches with the myocyte in relaxing solution [23]. Resting sarcomere length (measured using Aurora HVSL.901A video analysis software at 200 Hz) was set to 1.95–2.05 μm in relaxing solution. Clampex (pClamp, version 10.3, Molecular Devices) was used to record data and control the motor and perfusion systems.

2.4. Measurements of steady-state force-calcium relationship and dynamic response to fast stretches

After measurement of passive viscoelasticity, a fast perfusion system (SF-77B, Warner Instrument Corp) was used to activate each myocyte in the following sequence: pre-activating solution for 4–5 s, activating solution for 7 s, relaxing solution for 5 s (Fig. 1). During steady Ca^{2+} activation the cell was released by 10% of resting cell length (CL), then re-stretched 40 ms later, to help stabilize the sarcomeres. After 1–2 s, when force recovery from the release/restretch was complete, the cell was stretched by 0.5%, 1%, or 2% of CL in 10 ms, and held there for 1s. This stretch was slower than we used previously (2 ms [23]), but was done to minimize cellular damage, as the myocytes appear less robust at 37 °C than at the temperature of 15 °C used previously. After selecting for data quality and noise levels, we have $n = 5$ traces where 0.5% and 2% stretch was applied, and $n = 11$ traces where a 1% stretch was applied, due to more experiments being performed with 1% stretch protocol. The activation protocol was repeated for a range of calcium concentrations. Any decline in isometric force development was measured and corrected for using a reference activating solution (pCa 5.96 or 5.81) applied at intervals. The experiment was terminated when isometric force had declined by more than 20% from the first activation; this usually occurred after about 30 activations. When determining the force-calcium relation for SL = 1.8 μm, 2.0 μm or 2.2 μm, the SL was set with the myocyte in relaxing solution. Since 1.8 μm is below the slack length of the sarcomere (∼1.9μm), to set the SL to be 1.8 μm in the activated cell, the relation between SL and cell length was first determined in the relaxed myocyte using a series of length steps over the SL range 1.9 to 2.2 μm, then the cell was shortened to the length that would allow the sarcomeres to shorten during activation to SL = 1.8μm, as estimated from extrapolation of the SL-cell length relation.

2.5. Measurements of dynamic response to constant velocity shortening

Methods for this protocol were identical to those for the dynamic response to fast stretches, except for the change in cell length applied. During steady Ca^{2+} activation the cell was shortened by 7–11% of resting cell length (CL) starting from a sarcomere length of 2.3 μm, with a ramp of duration 100–300 ms, that is, with a velocity of 0.54 to 2.53 μm/s.

3. Results

We develop our multi-scale model of cardiac contraction by sequentially adding model complexity and experimental data, starting with the passive force, then steady-state activation, velocity-dependent dynamics, isometric twitch kinetics, and finally whole-organ contraction.

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