Load-Dependency in Mechanical Properties of Subepicardial and Subendocardial Cardiomyocytes

Anastasia Khokhlova[1,3], Gentaro Iribe[4], Olga Solovyova[1,2,3]

[1]Institute of Immunology and Physiology, Russian Academy of Sciences,
[2]Institute of Mathematics and Mechanics, Russian Academy of Sciences,
[3]Ural Federal University, Yekaterinburg, Russia,
[4]Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Abstract

Electrical and mechanical properties of myocardium vary transmurally in the left ventricular wall. Regional differences in the mechanical environment of cardiomyocytes may potentially contribute to this heterogeneity due to mechano-electric feedback. In the present study, we investigate transmural differences in active and passive tensions at different preloads between the subepicardial (EPI) and subendocardial (ENDO) cells isolated from mouse left ventricle, using our recently developed single cell stretch method where each cell end was held by a pair of carbon fibers to apply various extent of preload to the cells. To predict underlying mechanisms of the transmural differences, we used our electromechanical EPI and ENDO cell models to simulate experimentally obtained results.

Wet experiments showed that both passive and active tensions at different preloads are higher in ENDO cardiomyocytes, indicating higher stiffness and contractility in ENDO cells compared to EPI cells.

Our mathematical models reproduce experimental results, suggesting differences in the kinetics of cross bridges and calcium-troponin C complexes in ENDO and EPI models may essentially contribute to the differences in mechanical properties between the cells.

1. Introduction

Subepicardial (EPI) and subendocardial (ENDO) cardiomyocytes of ventricular wall differ in their mechanical properties. It has been reported that sarcomere length (SL) – tension' relationship underlying Frank-Starling law is different between the EPI and ENDO cells isolated from rat, ferret and guinea pig ventricles [1, 2]. Slope of the SL – active tension relationship is steeper in ENDO cardiomyocytes. ENDO cells demonstrate greater unloaded shortening with slower time to peak shortening and larger relaxation time constant than EPI cells in guinea pig and canine hearts [3, 4, 5], indicating greater contractility of ENDO cells compared to EPI cells. However mechanisms of this heterogeneity are still not clear.

In this paper we present results on preload-dependency in the amplitude of active and passive tensions of ENDO and EPI cardiomyocytes isolated from mouse left ventricles. Our recently developed single cell stretch method was used to apply 8-11 % axial stretch to the cells from different transmural layers. We utilized our electromechanical ENDO and EPI cellular models to reproduce experimental data and to predict mechanisms which may underlie the heterogeneity in active tension amplitudes of the cells.

2. Methods

2.1. Experiments

All experiments were conducted in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan, and the study protocol was approved by the Animal Subjects Committee of Okayama University. ENDO and EPI ventricular myocytes were enzymatically isolated from hearts excised from C57BL/6 mice (aged 9-11 weeks) and stored in normal Tyrode solution. Cardiomyocyte isolation method is described elsewhere [6].

2.1.1. Experimental setup

The single myocyte stretch system is described in detail elsewhere [6]. Briefly, each cell end was held by a pair of carbon fibers (CFs) to the top and bottom surfaces of the cell to apply 8-11 % axial stretch to the cells. Each CF on the left side is mounted on a computer-controlled piezoelectric transducer (PZT). Both CFs receive the same control command to achieve identical CF position control for stretching the cell end in one direction. All

single cell experiments were performed at 1 Hz stimulation at room temperature.--

2.1.2. Length and force measurements

The detailed method for performing length and force measurements is described elsewhere [7]. Briefly, cell length signal (CF tip distance) and SL changes were recorded using the IonOptix equipment and software (IonOptix Corporation, Milton MA, USA). Active and passive forces (*F*) were calculated using the following equation:

$$= K \cdot (\Delta L_{CF} - \Delta L_{PZT}), \quad (1)$$

where ΔL_{CF} is the change in distance between left and right CFs, and ΔL_{PZT} is the change in PZT position. The parenthetical term on the right side of the equation indicates total carbon fiber bending. *K* is the combined stiffness of left CFs.

Calculated force was normalized to effective cross-sectional area. Effective cell cross-sectional area was calculated from measured cell width, assuming an elliptical shape of the cross section with a 3:1 ratio of long (measured cell width, *y*-direction) and short axis (estimated cell height, *z*-direction) [7].

Figure 1 shows representative auxotonic contractions from a range of preloads (end-diastolic SL_o: 1.8 to 2.0) and calculated tensions superimposed for different preloads.

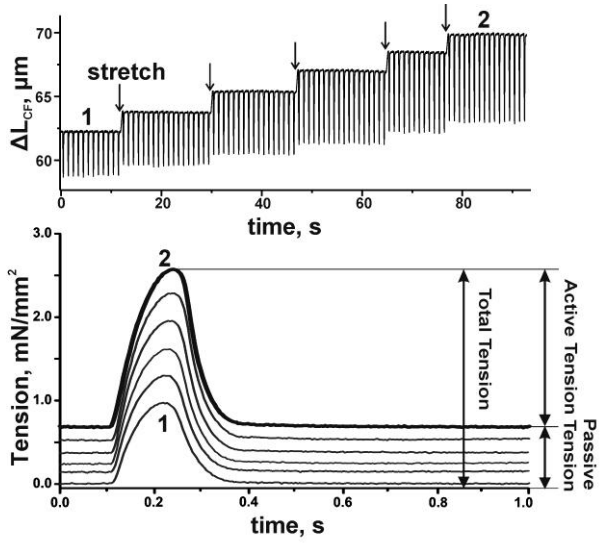

Figure 1. Top panel: representative experimental record of auxotonic twitches of mouse ventricular cardiomyocytes at different preloads. Bottom panel: cell tension (force per effective cross-sectional area) recordings plotted as a function of time, superimposed for different preloads.

All values are presented as means ± standard error of means (SEM). Student's unpaired t-test and two-way ANOVA were used for statistical analysis. Test was used as appropriate for statistical analyses and a p value of <0.05 was considered to indicate a significant difference between means.

2.2. Mathematical models

To predict mechanisms responsible for the transmural differences in mechanical properties, we utilized our electromechanical EPI and ENDO cell models [8, 9].

The EPI and ENDO models are based on the Ekaterinburg-Oxford mathematical model (EO model), which integrates the ionic and contractile mechanisms of excitation-contraction coupling in the guinea pig ventricular cardiomyocyte [10]. These EPI and ENDO cellular models allow one to reproduce specific transmural gradient in ionic currents, intracellular Ca^{2+} handling and mechanics for each cell type. As same as the experimental data, the EPI model produces significantly shorter action potential, faster Ca^{2+} transient and faster contractions with shorter time to peak contraction and lower rate constant of relaxation in both heavy-loaded isometric twitches and low-loaded isotonic contractions, as compared to the ENDO model [8, 9].

Passive tension in the models is calculated using the following equation:

$$_{passive} = \beta \cdot \left(e^{\alpha \cdot l(t)} - 1\right), \quad (2)$$

where *l(t)* is a relative change in the cell length against its slack length per sarcomere, β, α — model parameters.

Force generated by sarcomere ensemble which mainly determines active tension of the cell is defined by fraction of cross-bridges (Xbs) and depends on the velocity of sarcomere shortening/stretching:

$$_{CE} = \lambda \cdot N \cdot p(v), \quad (3)$$

where λ is the model parameter, *N* – fraction of Xbs. Function *p(v)* means dependence of the average Xb force on the sarcomere shortening/lengthening velocity $v(t) = l_1\dot{(t)}$, where $l_1(t)$ is the relative change in the SL against its slack length.

Fraction of Xbs *N(t)* depends itself on the sarcomere mechanics This mechanodependence of Xb kinetics assures a key molecular mechanism of the mechanoelectric feedback in cardiomyocytes:

$$\frac{dN}{dt} = k_{pv} \cdot M([CaTnC]) \cdot n_1(l_1) \cdot L_{oz} \cdot (1 - N) - k_{mv} \cdot N, \quad (4)$$

where k_{pv} and k_{mv} are velocity-dependent rate constants of Xb attachment and detachment, respectively, $n_1(l_1)$ is the probability of a myosin head "finding" a vacant site on the actin filament, L_{oz} is a normalized linear dependence of the sarcomere overlap zone on the SL.

The formation of the Xbs is affected by kinetics of CaTnC complexes. In the models we took into account cooperative end-to-end interaction between neighboring regulatory units (RUs) of the thin filament. We suggest that Ca^{2+} binding by TnC, located between neighboring RUs of the thin filament, induces end-to-end interactions between RUs, thus providing for opening of the additional actin sites for myosin head attachment [10, 11]. In the equation (4) *M[CaTnC]* indicates end-to-end interaction between neighboring RUs of the thin filament (figure 2):

$$M([CaTnC]) = \frac{(\frac{[CaTnC]}{[TnC]_{tot}})^{\mu}\cdot(1+0.6^{\mu})}{(\frac{[CaTnC]}{[TnC]_{tot}})^{\mu}+0.6^{\mu}}, \quad (5)$$

where *[CaTnC]* – concentration of CaTnC complexes, $[CaTnC]_{tot}$ – total TnC concentration, μ – parameter of cooperativity.

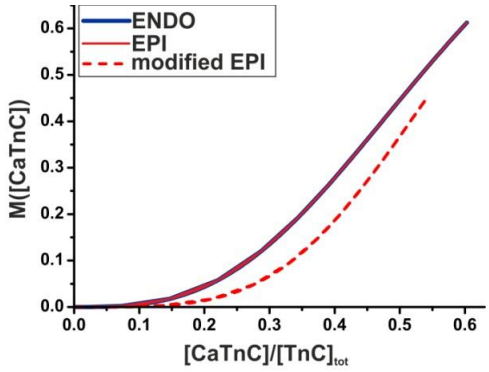

Figure 2. RU end-to-end interaction between neighboring RUs of the thin filament in the case where both of them are affected by the formation of respective CaTnC complexes.

Note that, in turn, the kinetics of the CaTnC is affected by mechano-dependent Xb kinetics making Ca^{2+} kinetics to be mechano-sensitive as well. Cooperative mechanisms of CaTnC kinetics as well as respective equations are identified and justified in our previous papers [10,11].

Thus, direct and feedback links between Ca^{2+} kinetics, cell shortening and force generation of the single cardiomyocyte are defined in the models and ensured from the cooperative mechanisms of myofilament Ca^{2+} activation.

3. Results

Figure 3 shows passive tension amplitude of ENDO and EPI cells at SL_o=2.0 μm in wet experiments and models. Despite we found no significant differences in passive tension amplitude between the cell types, ENDO cells tend to demonstrate greater amplitude compared to EPI cells (0.80 ± 0.13 vs. 0.56 ± 0.09 mN/mm^2), indicating tendency of greater stiffness in ENDO cardiomyocytes (figure 3, left panel). Models are in good qualitative consistency with the experimental data (0.49 vs. 0.17 mN/mm^2,figure 3, right panel). Scaling parameter β in the equation for passive tension in the models (2) was set to greater in 3 times for ENDO model than for EPI model.

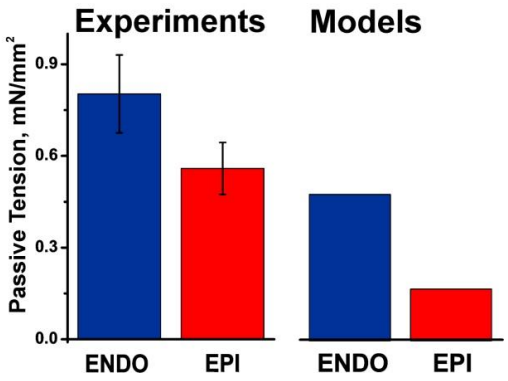

Figure 3. Passive tension of ENDO (n = 7) and EPI (n = 6) cells at SL_o=2.0 μm in wet experiments and models.

We found that ENDO cells demonstrated higher active tension amplitude in experiments at different preloads, indicating their greater contractility compared to EPI cells (at SL_o=1.8 μm: 0.80 ± 0.13 vs. 0.56 ± 0.09 mN/mm^2; at SL_o=2.0 μm: 1.64 ± 0.25 vs. 1.24 ± 0.13 mN/mm^2, figure 4). Stretch led to an increase in active tension amplitude in both ENDO and EPI cardiomyocytes (figure 4), however we found no significant differences in response to the stretch between the cell types.

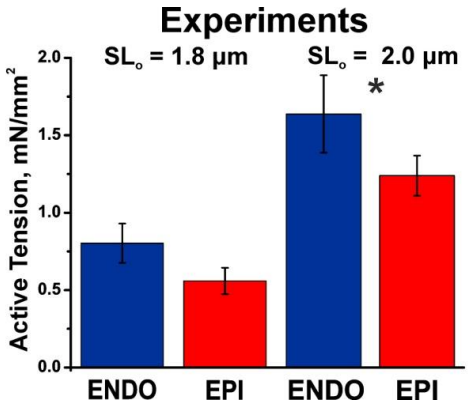

Figure 4. Active tension of ENDO (n = 7) and EPI (n = 6) cells at different preloads (SL_o=1.8 μm and 2.0 μm) in wet experiments.*p < 0.05 vs. active tension at SL_o=1.8 μm.

Initially, our ENDO and EPI models failed to reproduce transmural gradient in the active tension

amplitudes observed in experiments (ENDO vs. EPI, at SL_o=1.8 μm: 4.70 vs. 4.88 mN/mm^2; at SL_o=2.0 μm: 6.78 vs. 7.35 mN/mm^2 , figure 5). To solve this problem, we modified degree of nonlinearity for the RU end-to-end cooperativity in the EPI cells. An increase parameter of cooperativity μ in the equation (5) in 1.3 times in EPI model made RU end-to-end interaction lower in the EPI model than in ENDO model (figure 2). This modification resulted in pronounced regional gradient in active tension amplitude between the virtual cells (ENDO vs. EPI, at SL_o=1.8 μm: 4.70 vs. 3.70 mN/mm^2; at SL_o=2.0 μm: 6.78 vs. 5.90 mN/mm^2, figure 5). Thus, our modeling results suggest that different intracellular interactions between the kinetics of Xb and CaTnC may underlie heterogeneity in active force amplitude between the EPI and ENDO cardiomyocytes.

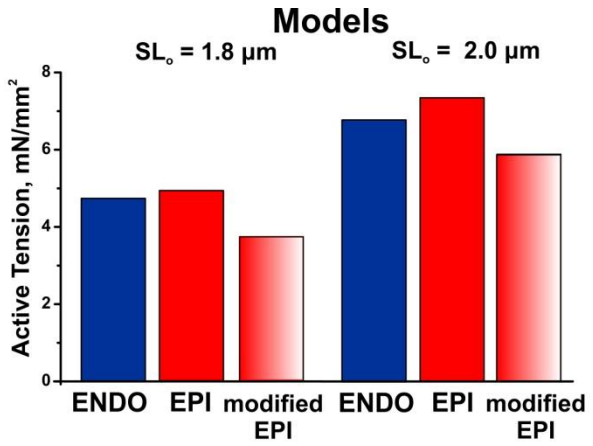

Figure 5. Active tension of ENDO and EPI cells at different preloads (SL_o=1.8 μm and 2.0 μm) in models.

4. Conclusions

The aim of the study was to investigate the load-dependency in tension amplitudes in EPI and ENDO cardiomyocytes using combination of our new experimental method and modeling approach. We showed that stretch leads to an increase in active tension amplitude in both cell types, but extent of these preload-dependent changes were not different between the EPI and ENDO cells.

We also found that ENDO cells tend to demonstrate greater stiffness and contractility at different preloads compared to EPI cardiomyocytes. Our mathematical models suggest that differences in the kinetics of cross bridges and calcium-troponin C complexes between the ENDO and EPI models may underlie the differences in mechanical properties between the cells.

Acknowledgements

Wet experiments are supported by Russian Basic Research Foundation (14-01-31134) and JSPS KAKENHI (26282121) Simulations are supported by the Russian Science Foundation (#14-35-00005).

References

[1] Cazorla O., Le Guennec J-Y., White E. Length–tension relationships of subepicardial and subendocardial single ventricular myocytes from rat and ferret hearts. Journal of molecular and cellular cardiology 2000; 32 (5): 735–744.

[2] Cazorla O, Pascarel C., Garnier D., Le Guennec J.Y. Resting tension participates in the modulation of active tension in isolated guinea pig ventricular myocytes. J Mol Cell Cardiol 1997; 29: 1629-1637.

[3] Bryant SM, Shipsey SJ, Hart G. Regional differences in electrical and mechanical properties of myocytes from guinea-pig hearts with mild left ventricular hypertrophy. Cardiovasc Res 1997;35:315-323.

[4] Wan X, Bryant SM, Hart G. A topographical study of mechanical and electrical properties of single myocytes isolated from normal guinea-pig ventricular muscle. J Anat 2003;202:525-536.

[5] Cordeiro J.M., Greene L., Heilmann C., Antzelevitch D., Antzelevitch C. Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. Am J Physiol Heart Circ Physiol 2004; 286: H1471-1479.

[6] Iribe G., Kaneko T., Yamaguchi Y., Naruse K. Load dependency in force-length relations in isolated single cardiomyocytes. Prog Biophys Mol Biol 2014; 115(2-3): 103-114.

[7] Iribe G., Helmes M., Kohl P. Force-length relations in isolated intact cardiomyocytes subjected to dynamic changes in mechanical load. Am J Physiol Heart Circ Physiol 2007; 292: H1487-1497.

[8] Vasilyeva AD, Solovyeva OE. Electromechanical coupling in cardiomyocytes from transmural layers of guinea pig left ventricle. Biophysics 2012;57:852-9.

[9] Vasilyeva A., Solovyova O. Modeling of heterogeneity in electrical and mechanical function of guinea pig ventricular myocytes. Comput Cardiol 2012;39:453-6.

[10] Sulman T, Katsnelson LB, Solovyova O, Markhasin VS. Mathematical modeling of mechanically modulated rhythm disturbances in homogeneous and heterogeneous myocardium with attenuated activity of na+ -k+ pump. Bull Math Biol 2008;70:910-49.

[11] Izakov V., Katsnelson L.B., Blyakhman F.A., Markhasin V.S., Shklyar T.F. Cooperative effects due to calcium binding by troponin and their consequences for contraction and relaxation of cardiac muscle under various conditions of mechanical loading. Circ Res 1991. 69: 1171-1184.

Address for correspondence.

Anastasia Khokhlova (a.khokhlova@iip.uran.ru)
Ural Federal University, 620002 Mira 19, Yekaterinburg, Russia