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RESIDUAL FORCE ENHANCEMENT IN MECHANICALLY ISOLATED HALF-SARCOMERES

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SUMMARY

Our goal was to test if half-sarcomeres produce force enhancement after stretch. Single myofibrils from rabbit psoas were placed in a temperature-controlled (10°C) bath, where half-sarcomeres were isolated using two pre-calibrated glass micro-needles. The force produced during activation of the half-sarcomere was measured by tracking the displacement of the micro-needles. The half-sarcomere length (HSL) variation was extracted by interpolating the displacement of the needles from the initial to the final distances measured from the Z-line to the center of the sarcomere. The steady-state forces after stretch were higher than the forces produced during the isometric contraction at the corresponding lengths. Force enhancement in half-sarcomeres cannot be attributed non-uniformity.

INTRODUCTION

The mechanism responsible for stretch-induced force enhancement [1] remains unclear in skeletal muscles. One of most used explanation – called sarcomere length non-uniformity - [2,3] has been recently challenged by studies that tracked individual sarcomeres from isolated myofibrils [4,5], and that used mechanically isolated single sarcomeres [6]. In the latter, our group noticed A-band displacements toward the sides of sarcomeres, suggesting that a non-uniform behavior between the two halves in a given sarcomere could produce force enhancement. However, even in absence of A-band displacement, some sarcomeres still produced force enhancement, which suggests that this phenomenon could also be attributed a mechanism residing in the half-sarcomere. The main goal of this study was to test if force enhancement can be elicited in isolated half-sarcomeres.

METHODS

Small bundles of the muscles were tied to wooden sticks, and chemically permeabilized using a standard protocol [7,8]. The muscles were incubated in rigor solution (pH = 7.0) for approximately 4 hours, after which they were transferred to a rigor:glycerol (50:50) solution for 20 hours. The samples were placed in a new rigor:glycerol (50:50) solution with the addition of a mixture of protease inhibitors and stored in a freezer (−20°C) for at least seven days. On the day of the experiment, a muscle sample was transferred to a fresh rigor solution and stored in the fridge for one hour before use. A small section of the sample was extracted (~1 mm3) homogenized in a rigor solution (pH = 7.0) using the following sequence: twice for 5 s at 7,500 rpm, and once for 3 s at 18,000 rpm. The homogenizing protocol produces a supernatant containing single myofibrils. This homogenate was transferred into an experimental chamber with the bottom made of a vacuum grease-sealed glass coverslip, placed on the stage of an inverted microscope. The chamber was filled with rigor solution, and the temperature was controlled at ~10°C. A myofibril was chosen based on its striation appearance, and either a single sarcomere or a half sarcomere was selected for mechanical experimentation. Using micromanipulators the single sarcomeres were captured by two pre-calibrated micro-needles that were pierced externally adjacent to each Z-lines, while the half-sarcomeres were pierced by one needle adjacent to the M-line, and the second externally, adjacent to the Z-line. The contrast between the micro-needles produces the darkest light peaks that allow for their centroids to be tracked by an algorithm using Image J software (NIH, USA). The half-sarcomere length variation was obtained by interpolating the displacement of the micro-needles from the initial to the final distances measured from the Z-line to the center of the sarcomere. The force produced during activation of the single and half-sarcomeres was measured considering the displacement of both micro-needles, as described in more details elsewhere [6].

Solutions

The rigor solution (pH 7.0) was composed of (in mM): 50 Tris, 100 NaCl, 2 KCl, 2 $MgCl_2$, and 10 EGTA. The activating (pCa^{2+} of 4.5) and relaxing (pCa^{2+} of 9.0) - pH 7.0- solutions contained (in mM): 20 imidazole, 14.5 creatine phosphate, 7 EGTA, 4 MgATP, 1 free Mg^{2+}, free Ca^{2+} in two concentrations adjusted to obtain pCa^{2+} of 4.5 (32mM) and 9.0 (1nM); KCl was used to adjust the ionic strength to 180 mM in all solutions

Protocol

Single sarcomeres (n=18) and half-sarcomeres (n=17) were immersed in relaxing solution for 1-2 s, then the solution was rapidly replaced by an activating solution using a double-barreled micropipette. When surrounded by the activating solution, the preparation from both groups contracted and produced force. After full force development single and half-sarcomeres were stretched by different magnitudes ranging from 15 – 36% of SL and HSL, in speeds ranging from 1.35 to 3.15 $\mu m \cdot s^{-1} \cdot HSL^{-1}$. After the end of the stretch, the myofibrils were held isometric for at least 5 s before being relaxed.

Data analysis

The force obtained after stretch, and at the isometric reference contractions were compared between single and half-sarcomeres. A two-way-mixed-factorial analysis of covariance (ANCOVA) for repeated measures was used to compare the forces produced by the two groups (single and half-sarcomere) in 2 conditions (isometric and after stretch), considering amount of stretch as a co-variant. The significance level for all statistical tests was set at $P < 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the force-length relationship constructed based on the isometric forces produced by half- and single sarcomeres. In both cases, the steady-state force after stretch was significantly higher than the force produced during the isometric contraction at the corresponding SL and HSL. The forces produced by half and single sarcomeres after stretch were significantly higher than the predicted by the force and length relationship, nonetheless they were not different from one another.

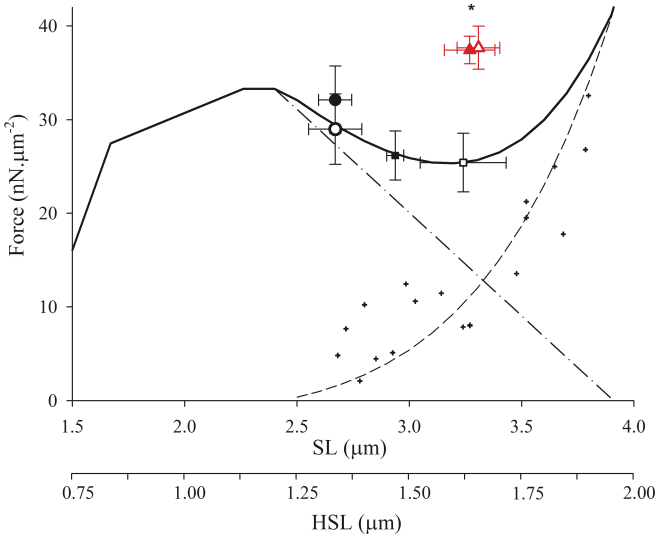

Figure 1 – Force-length relationship built based on isometric contractions produced by single (black closed symbols) and half-sarcomeres (black open symbols). The dashed line corresponds to the passive force produced by deactivated myofibrils, the dash-dotted line corresponds to the descending limb of the force-length relationship, the continuous line corresponds to theoretical force-length relation based on the known length of the thick and thin filaments of the rabbit psoas muscles. In red are the forces produced after a stretch was imposed to single (closed-triangle) and half sarcomeres (opened-triangle). The circles are mean force values from isometric contractions close to the plateau, while the squares were performed at longer SL and HSL. (*) Significantly different from the predictive correspondent isometric force, $P < 0.05$.

This is the first study that shows the feasibility to mechanically isolate one half of a single sarcomere. Our results directly show that half-sarcomeres produce the same amount of isometric force as individual sarcomeres, and that the force is higher when both, single and half-sarcomeres are stretched after activation than their correspondent isometric force at the same HSL, implicating a mechanism other than non-uniformity in force enhancement in half-sarcomeres. We speculate that the potential mechanism for force enhancement in half-sarcomeres is attributable to an increase in the number of attached cross-bridges [7] and/or stiffening of titin molecules upon calcium activation [9].

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