Supporting Information

Caremani et al. 10.1073/pnas.1525057113

SI Methods

Sample Preparation. Male rats (Wistar, 230–280 g) were anesthetized with isoflurane [5% (vol/vol)]. The heart was rapidly excised, placed in a dissection dish, and retrogradely perfused with a modified Krebs–Henseleit solution (composition, in mM: NaCl, 115; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 0.5; glucose, 10), containing 20 mM 2,3-butanedione monoxime [which has been shown to protect the myocardium during dissection (26)], and oxygenated with 95% O_2 and 5% CO_2 (pH 7.4). A thin, unbranched, and uniform trabecula was dissected from the right ventricle using a stereomicroscope. The axial orientation of the trabecula was determined by means of the attachment of the two extremities using a double titanium hook. The width (w) and the height (h) of the trabecula were measured by an eyepiece with a graduate scale (10 μm per division) with the trabecula stretched to be just taught. The trabecula was mounted horizontally in a temperature-controlled trough (1.2-mL volume) attaching the triangular vertex of the double hook to the lever arms of a capacitance gauge force transducer (valve side) and a loudspeaker motor servosystem (wall side). In this way, the trabecula was aligned with the transducer levers, and, during contraction, the transversal movements were minimized. The characteristics of the force and length transducers have been already reported (ref. 27 and reference therein). The through was perfused at 1.2 mL/min, and the temperature of the solution was maintained at 27 °C. The sarcomere length (SL) was set at 2.2 μm at rest by using a 40× objective, and L_0, the trabecula length at SL of 2.2 μm, was estimated as the distance between the double-hook attachment at the wall side and the attachment of the trabecula to the valve, at the valve side. The cross-sectional area was calculated by assuming an elliptical cross-section. The dimensions of the preparations were as follows (mean ± SD, $n = 10$): w, 83–350 μm (211 ± 97 μm); h, 63–200 μm (97 ± 37 μm); cross-sectional area, 4,800–47,100 μm^2 (17,660 ± 13,300 μm^2); L_0, 1.0–4.2 mm (2.7 ± 0.7 mm).

Experimental Protocol. Trabeculae were electrically stimulated by means of two platinum plate electrodes, 4 mm apart, with bipolar pulses of 0.5-ms duration and amplitude 1.5× the threshold voltage. Measurements with specific mechanical protocols were made at the steady state of the contraction–relaxation cycle during electrical pacing at 0.5 Hz. A striation follower was used to record SL changes in a segment of 0.7–1.5 mm selected along the central region of the preparation (10). The relation between the force peak (T_P) and the SL was determined by changing the length of the trabecula with the micromanipulator carrying the loudspeaker motor and measuring the SL with the outputs of either spot delimiting the segment selected for the striation follower signal (10). The relation between SL and trabecula length was linear up to SL of 2.2 μm and then deviated downward (Fig. S1).

To determine the isotonic velocity transient, the control was switched from length to force feedback at 95% of the peak of the isometric contraction, and 1 ms later a stepwise reduction in force, rise time of ∼200 μs, was imposed by using as a command signal the output of an integrated circuit that generated steps to preset fractions of T_P (8, 19). When the isotonic shortening attained ∼60 $nm \cdot hs^{-1}$, the control was shifted back to length feedback to terminate the twitch in fixed-end mode at the new length. In force-clamp mode, the direct signal of force was used in the feedback, and the velocity signal was taken from the motor position signal. At any clamped force, several trials were necessary to adjust the gains of direct, velocity and integrative amplifiers and minimize the duration to optimize the shape of the step. The isotonic velocity transient (range, 0.2–0.8 T_P) was elicited at different SLs (1.9 and 2.2 μm) and at different extracellular Ca^{2+} concentrations (1 and 2.5 mM).

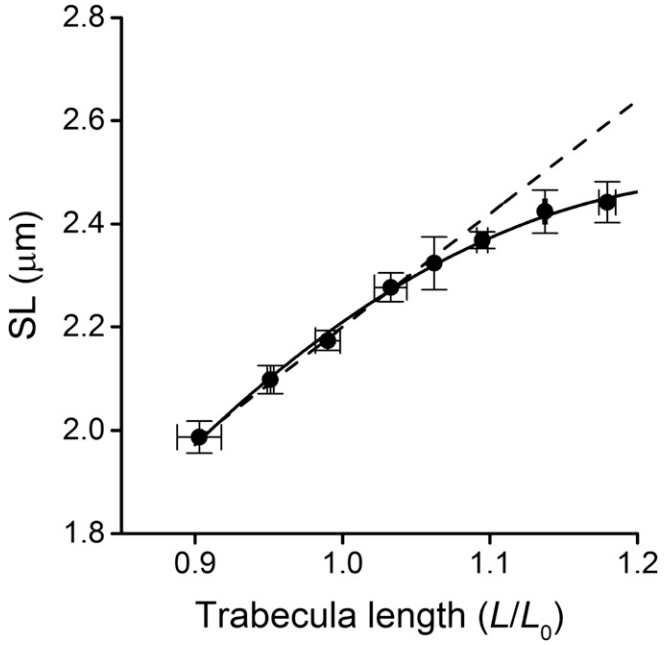
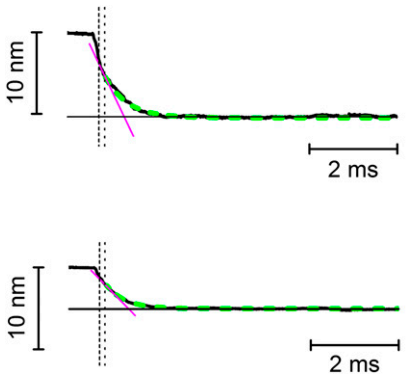


Fig. S1. Relation between SL and trabecula length (relative to L_0) in a quiescent trabecula. Points are the mean ± SD (two trabeculae with L_0 of 1.1 and 2.5 mm). A progressive deviation of SL from the value expected from the linear relation (dashed line) appears with the rise of passive force, as part of the imposed lengthening is taken by the compliant ends of the trabecula. The continuous line is a parabolic fit to data.



T/T_P	$r_{2,exp}$ (ms^{-1})	$r_{2,tg}$ (ms^{-1})
0.52 ± 0.01	2.75 ± 0.49	2.55 ± 0.74
0.76 ± 0.01	1.32 ± 0.09	1.31 ± 0.13

Fig. S2. Estimate of the rate of phase 2 velocity transient. Shortening transient elicited by a force step to ~0.5 T_P (*Upper*) and to ~0.75 T_P (*Lower*) after subtraction of phase 4 shortening. r_2 is estimated either as the reciprocal of the time between $t_{1/2}$ (vertical dashed line as in Fig. 2*C*) and the abscissa intercept of the tangent to the initial part of phase 2 shortening (magenta line as in Fig. 2*C*), $r_{2,tg}$, or from the exponential fit (green dashed line) of the trace starting from the end of the force step (dotted vertical line), $r_{2,exp}$. As shown in the table, the two methods gave similar results (mean ± SEM from four trabeculae).

Other Supporting Information Files

Dataset S1 (XLSX)