

Tension generation and relaxation in single myofibrils from human atrial and ventricular myocardium

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Abstract Fast solution switching techniques in single myofibrils offer the opportunity to dissect and directly examine the sarcomeric mechanisms responsible for force generation and relaxation. The feasibility of this approach is tested here in human cardiac myofibrils isolated from small samples of atrial and ventricular tissue. At sarcomere lengths between 2.0 and 2.3 μm , resting tensions were significantly higher in ventricular than in atrial myofibrils. The rate constant of active tension generation after maximal Ca^{2+} activation (k_{ACT}) was markedly faster in atrial than in ventricular myofibrils. In both myofibril types k_{ACT} was the same as the rate of tension redevelopment after mechanical perturbations and decreased significantly by decreasing $[\text{Ca}^{2+}]$ in the activating solution. Upon sudden Ca^{2+} removal, active tension fully relaxed. Relaxation kinetics were (1) much faster in atrial than in ventricular myofibrils, (2) unaffected by bepridil, a drug

that increases the affinity of troponin for Ca^{2+} , and (3) strongly accelerated by small increases in inorganic phosphate concentration. The results indicate that myofibril tension activation and relaxation rates reflect apparent cross-bridge kinetics and their Ca^{2+} regulation rather than the rates at which thin filaments are switched on or off by Ca^{2+} binding or removal. Myofibrils from human hearts retain intact mechanisms for contraction regulation and tension generation and represent a viable experimental model to investigate function and dysfunction of human cardiac sarcomeres.

Keywords Cardiac sarcomere · Calcium regulation · Myosin · Titin · Bepridil · Inorganic phosphate

Introduction

Current approaches to relate cardiac myofibril properties with disease states are often oriented toward monitoring changes in protein expression and chemical activity that may reflect, but do not measure, changes in functionally relevant parameters. Skinned strips [1–5] and skinned myocytes [6–8] isolated from relatively large samples of human cardiac muscle are the “conventional” preparations used to measure changes in fundamental indices of muscle performance accompanying alterations of sarcomeric proteins. A significant advancement in the ability to directly measure changes in the mechanical performance of cardiac muscle accompanying alterations of sarcomeric proteins in humans can be offered by the use of single myofibrils, a preparation that may be obtained in large amounts even from small cardiac biopsies [9]. Single myofibrils are the smallest units of the contractile apparatus of striated muscle that retain the organized myofilament lattice and entire

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ensemble of associated proteins. Miniaturized mechanical methods measuring myofibril force have been used for several years (e.g., [10–12]). The small size and short diffusion distance of myofibrils allowed us to exploit recent advances in rapid perfusion switching techniques [13] to investigate fast kinetic events related to cross-bridge action and regulation [14, 15]. Though the myofibril, as a model for mechanical experiments, is superior in several important ways to larger muscle preparations, mostly animal models have been used to date in muscle research at the single myofibril level.

In this study, the feasibility of using single myofibrils from human cardiac biopsy samples for mechanical experiments is established and the sarcomeric mechanisms underlying passive and active force generation and relaxation are dissected and compared in atrial and ventricular muscle.

Materials and methods

Patients and preparation of myofibrils

The investigation conforms to the principles outlined in the Declaration of Helsinki. Transmural left ventricular needle biopsy samples (3–5 mm³) were obtained for microscopic analysis from informed patients undergoing corrective heart surgery, according to a protocol approved by the local Ethics Committee. Among the excess samples, three patients (age 45–75 years; 1 ♀ and 2 ♂) were selected on the basis of normal left ventricular contraction patterns and absence of hypertrophy. A fourth ventricular sample came from a healthy heart donor (♀, 26 years). Left atrial biopsy samples, routinely excised during cardioplegia, were obtained from informed patients, in sinus rhythm and without atrial dilatation, undergoing coronary artery bypass surgery (2 ♀ and 2 ♂; age 41–74 years). All patients, but the donor, had β -blockers and nitrates in their therapy; four of them also took ACE inhibitors and diuretics. Unless otherwise stated, data from different samples of each myofibril type were pooled together.

Bioptic samples were placed in a Petri dish covered with Sylgard, cut into thin strips under a stereomicroscope, and pinned down in ice-cold rigor solution containing (mM) NaCl 132, KCl 5, MgCl₂ 1, TRIS 10, ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) 5, (pH 7.1). The strips were incubated for 3 h in the same solution added with 1% Triton X100. Triton was then removed and the strips were either immediately homogenized in rigor solution to produce myofibril suspensions or frozen in liquid nitrogen and stored at –80°C to be homogenized within a few months. Myofibril suspensions, stored at 0–4°C, were stable and were used for up to 5 days

after preparation. No relevant difference was found between myofibrils prepared from fresh or frozen cardiac strips.

All solutions to which the samples and myofibrils were exposed contained a cocktail of protease inhibitors including leupeptin (10 μ M), pepstatin (5 μ M), phenylmethylsulphonylfluoride (200 μ M), E64 (10 μ M), NaN₃ (500 μ M), and dithioerythritol (2 mM).

Apparatus and experimental protocol

In the present experiments we used previously published techniques to measure and control the force and length of isolated myofibrils activated and relaxed by fast solution switching [11, 14, 15]. Briefly, a small volume of the myofibril suspension was transferred to a temperature-controlled chamber (15°C) filled with relaxing solution (pCa 8.0) and mounted on an inverted microscope. Selected preparations (single myofibrils or bundles of few myofibrils, 25–80 μ m long, 1–4 μ m wide) were mounted horizontally between two glass microtools. One tool was connected to a length-control motor that could produce rapid (<1 ms) length changes. The second tool was a calibrated cantilevered force probe (2–6 nm nN⁻¹; frequency response 2–5 kHz). Force was measured from the deflection of the image of the force probe projected on a split photodiode. Average sarcomere length and myofibril diameter were measured from video images (ca. \times 1,800).

The initial sarcomere length of the preparations was set around 2.2 μ m. Myofibrils were activated and relaxed by rapidly translating the interface between two flowing streams of solution of different pCa across the length of the preparation. The solution change took place with a time constant of 2–3 ms and was complete in <10 ms.

Solutions

Activating and relaxing solutions, calculated as previously described [14], were at pH 7.00 and contained 10 mM of total EGTA (CaEGTA/EGTA ratio set to obtain different pCa values in the range of 8.00–3.50), 5 mM of MgATP, 1 mM of free Mg²⁺, 10 mM of 3-(*N*-morpholino)propane-sulfonic acid, propionate, and sulfate to adjust the final solution to an ionic strength of 200 mM and monovalent cation concentration of 155 mM. Creatine phosphate (10 mM) and creatine kinase (200 U ml⁻¹) were added to all solutions. Unless otherwise stated, contaminant inorganic phosphate (P_i) from spontaneous breakdown of MgATP and CP was reduced to <5 μ M by a P_i scavenging system (purine–nucleoside–phosphorylase with substrate 7-methylguanosine; [14]). All solutions contained the cocktail of protease inhibitors mentioned above.

Results

Passive tension

Sarcomere lengths of slack human cardiac myofibrils in relaxing solution ranged between 1.8 and 2.0 μm for both atrial and ventricular preparations. At sarcomere lengths around 2.2 μm , however, ventricular myofibrils generated significantly higher passive tension than atrial myofibrils (see Table 1), suggesting that resting stiffness differs in the two myofibril types. To better investigate this, quasisteady-state sarcomere length–resting tension relations were determined in relaxing solution for ten atrial and seven ventricular myofibrils (Fig. 1). Ramp elongations of different extent (10–30% of the slack myofibril length) were applied to the preparations. Sarcomere length and resting tension were measured several seconds after each length change, when most of stress relaxation was over (see representative tracings in Fig. 1a,b). The relatively large difference in the average sarcomere length–resting tension relations of the two myofibril types (Fig. 1c) confirmed that ventricular myofibrils exhibit higher resting stiffness than atrial myofibrils. The result is consistent with the observation that the atria of large mammals express higher levels of the compliant (N2BA) titin isoform than the ventricles [16].

Active tension generation

Examples of myofibril active tension responses to maximal Ca^{2+} activation by fast solution switching are shown in Fig. 2 and average data are given in Table 1. Values of maximal isometric tension (P_0) were in the upper range of those usually reported for cardiac preparations (see “Discussion”) and did not differ significantly between atrial and ventricular myofibrils.

In both myofibril types the rate constant of tension activation (k_{ACT}) after stepwise increase in $[\text{Ca}^{2+}]$ was the same as that of tension redevelopment (k_{TR}) after stepwise changes in length (release–restretch) applied to the myofibril under conditions of steady activation (Table 1). This suggests that k_{ACT} is limited by the rate at which detached or weakly bound cross bridges enter the force-generating states rather than by the rate at which the thin filament is switched on by Ca^{2+} binding to TnC.

As expected from the different myosin heavy-chain (MyHC) isoforms predominantly expressed by human atrial and ventricular myocardium [17], k_{ACT} and k_{TR} were markedly faster in atrial than in ventricular myofibrils (Table 1 and Fig. 2c,d). On average, the kinetics of force development did not differ significantly among myofibrils of the same type taken from different patients whereas the different kinetics of atrial and ventricular myofibrils were evident from comparisons between any populations of the two types (Fig. 2d).

Active tension, k_{ACT} , and k_{TR} strongly depended on the $[\text{Ca}^{2+}]$ in the activating solution (Fig. 3), demonstrating that contractile regulation of human cardiac myofibrils is intact. In both atrial and ventricular myofibrils the threshold for contractile activation occurred at between pCa 6.25 and 6.00 and half maximal activation was found at around pCa 5.60 (Fig. 3c). The parameters of the pCa–tension relation were very close to those previously reported for larger preparations of human skinned cardiac muscle [5]. Analysis of the kinetics of force development clearly showed that k_{ACT} (and k_{TR}) was strongly dependent on Ca^{2+} -activated force (Fig. 3d), as previously reported for experiments on various striated animal muscle preparations (for review, see [18]). In spite of the large difference in the absolute values of k_{ACT} and k_{TR} of human atrial and ventricular myofibrils, the relative effects of Ca^{2+} -activated force on the kinetics of

Table 1 Means (\pm SEM) of resting parameters, maximum active tension (P_0), and kinetic parameters for full tension generation and relaxation in human atrial and ventricular myofibrils

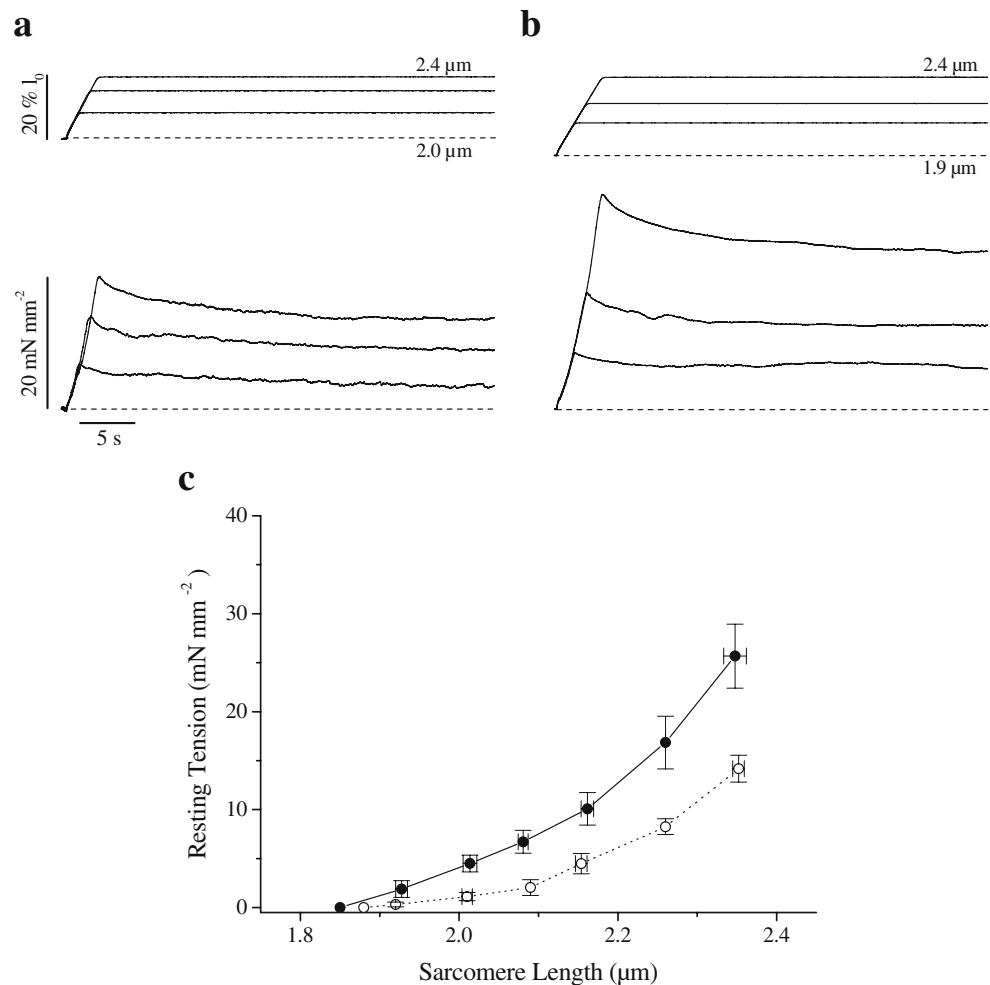
	Atrium	Ventricle	<i>P</i> value
Resting conditions			
Initial s.l. (μm)	2.20 \pm 0.01 (76)	2.19 \pm 0.01 (62)	N.S.
Passive tension (mN mm^{-2})	6.4 \pm 0.6 (76)	10.1 \pm 1.1 (53)	$p < 0.002$
Active tension generation			
P_0 (mN mm^{-2})	125 \pm 7 (52)	108 \pm 7 (53)	N.S.
k_{ACT} (s^{-1})	3.73 \pm 0.18 (54)	0.73 \pm 0.03 (62)	$p < 0.001$
k_{TR} (s^{-1})	3.55 \pm 0.19 (46)	0.68 \pm 0.03 (55)	$p < 0.001$
Full tension relaxation			
Slow phase duration (ms)	126 \pm 6 (47)	226 \pm 8 (47)	$p < 0.001$
Slow k_{REL} (s^{-1})	0.52 \pm 0.04 (47)	0.15 \pm 0.02 (47)	$p < 0.001$
Fast k_{REL} (s^{-1})	16.0 \pm 1.0 (47)	2.90 \pm 0.16 (47)	$p < 0.001$

Values in parentheses are the myofibril numbers (data pooled from all patient samples)

The *p* values were estimated by the Student *t* test.

Experimental conditions: 15°C; pCa of relaxing and activating solution, 8.00 and 3.50, respectively; [MgATP] 5 mM; and $[P_i] < 5 \mu\text{M}$

Fig. 1 Passive tension generation in human atrial and ventricular myofibrils. **a, b** Representative tension responses (*bottom traces*) of atrial (**a**) and ventricular (**b**) myofibrils to passive elongations (*top traces*) in relaxing solution (pCa 8.0). Initial myofibril length (l_0) just above the slack (*dashed line*, zero resting tension); temperature 15°C. In both preparations stress relaxation occurs at the end of the length ramp. **c** Average sarcomere length–resting tension relationships of ten atrial (*open symbols and dotted line*) and seven ventricular (*closed circles and continuous line*) myofibrils. Vertical and horizontal bars are SEM. Resting tension and mean sarcomere length were measured under quasisteady-state conditions, i.e., about 30 s after the end of elongation when most of stress relaxation was over



force generation were similar. In both types of myofibrils, k_{ACT} (and k_{TR}) increased more than five times from low to maximal Ca^{2+} -activated force. The results suggest that in human myocardium, like in other muscle types [18], the degree of Ca^{2+} activation of thin filaments limits the probability of the strong attachment of cross bridges to actin.

Active tension relaxation

As shown in Fig. 2a,b, active tension fully relaxed with step reduction of $[\text{Ca}^{2+}]$ below the contraction threshold. The kinetics of full tension relaxation in human atrial and ventricular myofibrils are shown on a faster timescale in Fig. 4a. As previously described in other myofibril types [9, 12, 15], the time course of complete force relaxation in human cardiac myofibrils was biphasic, starting with a slow, nearly linear force decay followed, after a “shoulder”, by a fast, exponential relaxation phase. It was shown [9, 12] that the slow linear force decay occurs under isometric sarcomere conditions while the fast exponential phase follows the “give” of one or more sarcomeres and is dominated by intersarcomere dynamics.

Relaxation kinetics were much faster in atrial myofibrils than in ventricular myofibrils. The linear relaxation phase lasted twice as long and its rate constant (slow k_{REL}) was three to four times slower in ventricular myofibrils than for atrial myofibrils; the difference in the rate constant of the final fast phase of relaxation (fast k_{REL}) between atrial and ventricular preparations was even larger (see Table 1). In spite of some variability, relaxation parameters did not differ significantly among myofibril populations of the same type obtained from different patients whereas they differed markedly between any populations of atrial and ventricular myofibrils (Fig. 4b–d).

In both myofibril types, slow k_{REL} was several times slower than k_{ACT} measured at maximal activation (Table 1). However, in both preparations, slow k_{REL} was almost undistinguishable from k_{ACT} measured at low $[\text{Ca}^{2+}]$ -activated force levels (see Fig. 3d). At relative tension around 0.2 P_0 , in fact, mean k_{ACT} was 0.46 ± 0.06 and $0.11 \pm 0.02 \text{ s}^{-1}$ for atrial ($n=9$) and ventricular ($n=11$) myofibrils, respectively; these values did not differ significantly from the slow k_{REL} values reported in Table 1 (0.52 ± 0.04 and $0.15 \pm 0.02 \text{ s}^{-1}$ for atrial and ventricular myofibrils, respec-

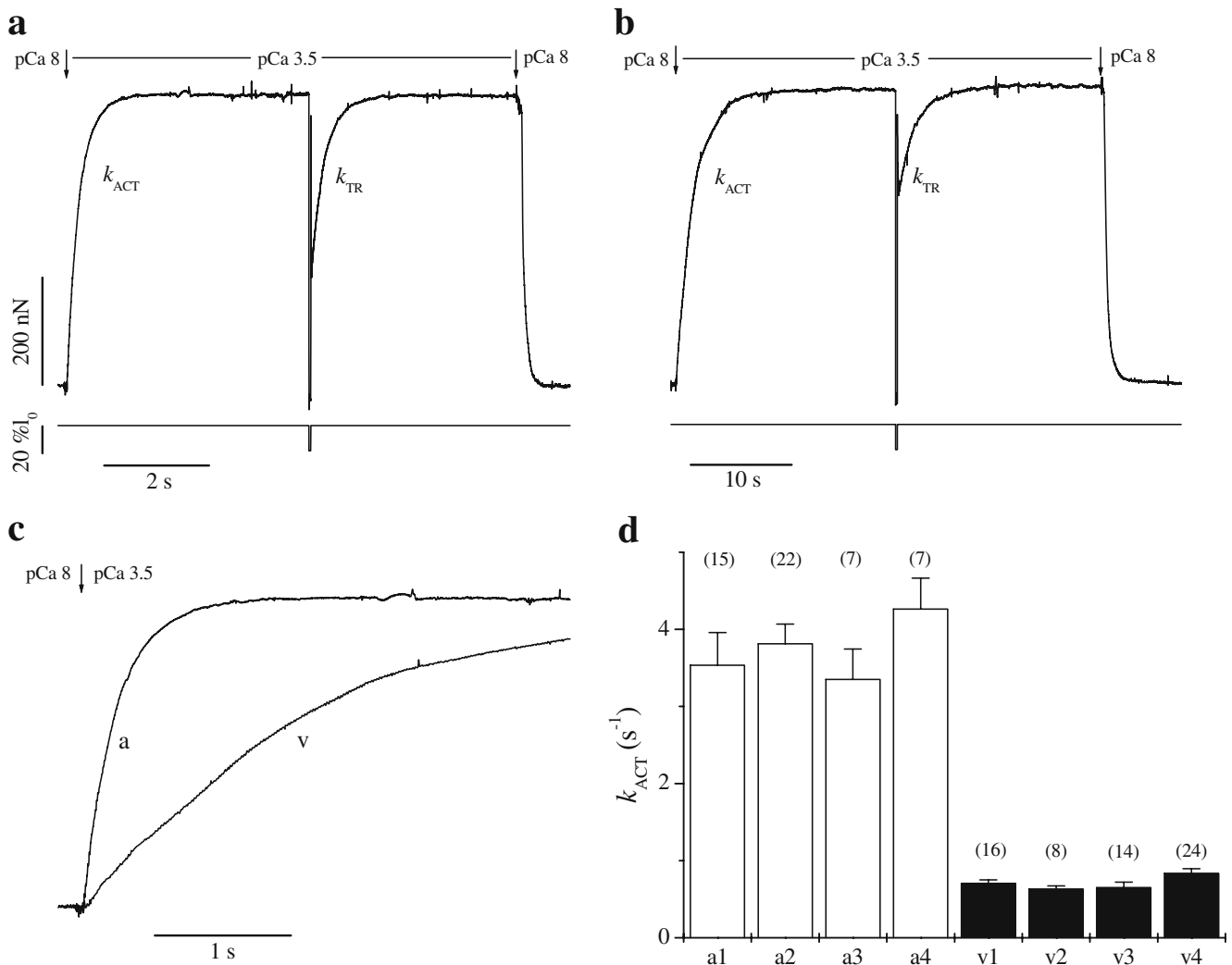


Fig. 2 Active tension generation in human cardiac myofibrils. **a, b** Representative force responses (*top traces*) of atrial (**a**) and ventricular (**b**) myofibrils maximally activated and fully relaxed by fast solution switching (pCa changes at *arrows* as indicated). Initial sarcomere length 2.2 μ m; temperature 15°C. Fast length changes (*bottom traces*) are applied to the myofibrils under conditions of steady tension generation. k_{ACT} is the rate constant of tension generation after fast Ca^{2+} activation; k_{TR} is the rate constant of tension redevelopment after

the release–restretch. **c** The time course of tension activation of the atrial (*a*) and ventricular (*v*) myofibrils shown in **a** and **b** are superimposed on a faster time base and after normalization for maximal tension. **d** Mean values of k_{ACT} of the atrial (*white columns*) and ventricular (*black columns*) myofibrils obtained from each patient. *Bars on top of columns* are SEM; the number of myofibrils for each patient is given in *parentheses*

tively). The similarity between slow k_{REL} and k_{ACT} at low Ca^{2+} activation seems to be consistent with both kinetic parameters reflecting cross-bridge kinetics. During tension generation at low Ca^{2+} activation and during full relaxation after Ca^{2+} removal to subthreshold level, the probability that cross bridges enter the force-generating transition is fairly low and the apparent cross-bridge kinetics are dominated by the rate at which attached cross bridges leave the force-generating states [9].

The idea that relaxation kinetics of human cardiac myofibrils reflect cross-bridge detachment rates rather than the rate at which thin filaments are switched off by Ca^{2+} removal is further supported by results shown in Fig. 5 and

Table 2. Bepridil, a Ca^{2+} sensitizer [19] that reduces the rate of dissociation of Ca^{2+} from the regulatory site of cardiac TnC [20], did not slow force relaxation kinetics of human atrial myofibrils (Fig. 5d). Relaxation rates, instead, were markedly accelerated by slight increases in inorganic phosphate concentration ($[P_i]$) that are thought to favor cross-bridge detachment through reversal of the power stroke (for review, see [18]). Removal of the P_i mop from the perfusing solutions (see “Materials and methods”) increased the contaminant P_i levels from $<5 \mu$ M to about 200 μ M. As shown in Table 2, 200 μ M P_i significantly shortened the duration of the slow relaxation phase and accelerated both slow k_{REL} and fast k_{REL} of human atrial myofibrils.

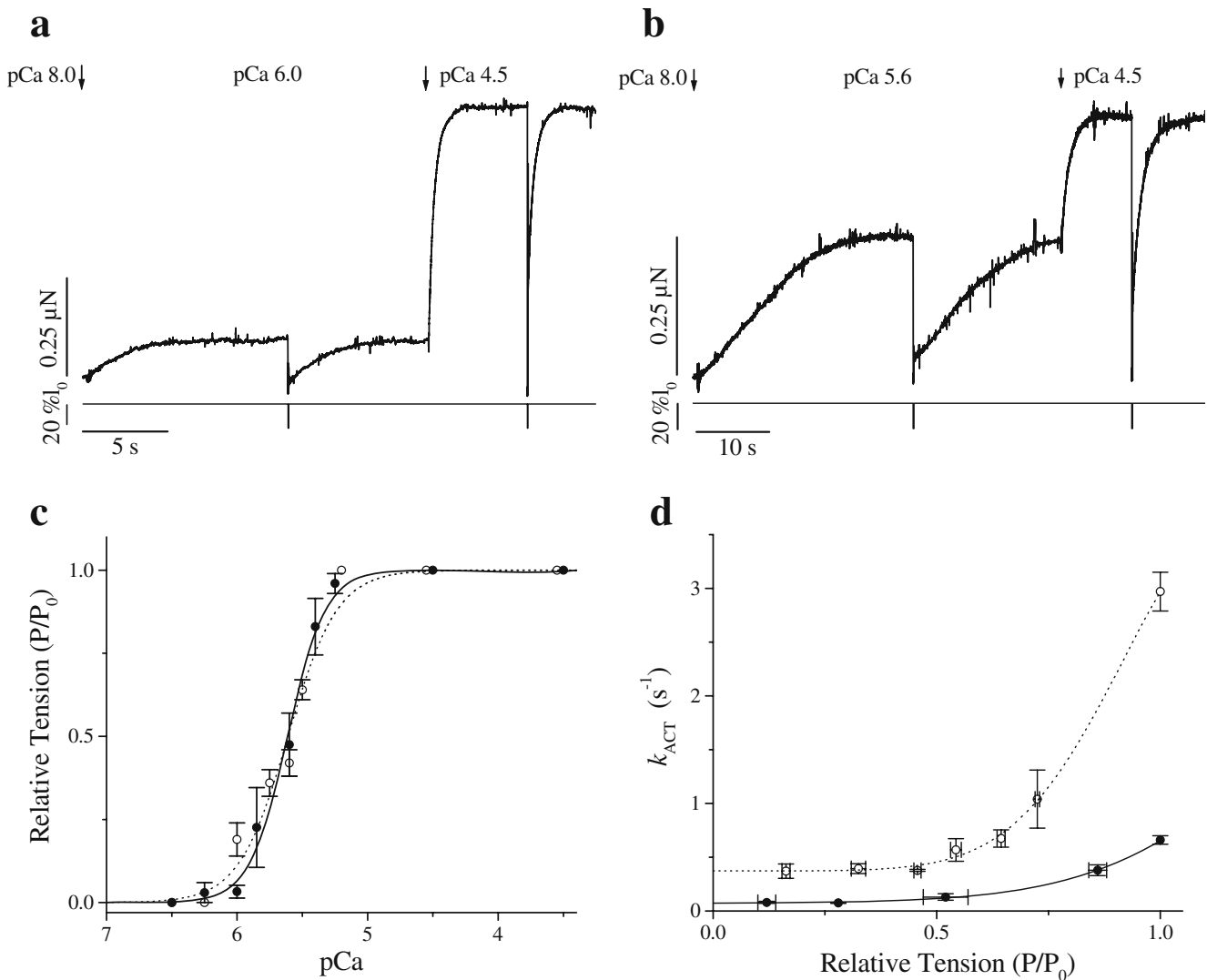


Fig. 3 Ca^{2+} -dependence of tension generation in human cardiac myofibrils. **a, b** Representative tension responses of atrial (**a**) and ventricular (**b**) myofibrils to submaximal and maximal Ca^{2+} activation. The *first arrow* marks the switching of myofibril perfusion from relaxing solution to submaximally activating solution; the *second arrow* marks the switching from submaximally activating solution to maximally activating solution (pCa changes as indicated). At each level of Ca^{2+} activation, a release–restretch (*bottom trace*) is applied to the preparation under steady-state condition of tension generation to measure k_{TR} . In each case the kinetics of tension generation after the Ca^{2+} activations are quite the same as those after the mechanical perturbations; both kinetics are extremely sensitive to $[\text{Ca}^{2+}]$. **c** Average pCa–tension relationships of atrial (*open circles and dotted line*) and ventricular (*closed circles and continuous line*) myofibrils.

Discussion

These results show that single myofibrils from small human cardiac biopsies retain intact mechanisms of contraction regulation and force generation; they may be viable experimental models to investigate function and dysfunction of human cardiac sarcomeres. The large differences

observed between atrial and ventricular myofibril mechanics and kinetics are at least partly expected from known differences in the sarcomeric protein pattern expressed by the two types of working myocardium. The kinetics of force activation and relaxation after sudden Ca^{2+} increase and decrease by fast solution switching in both types of cardiac myofibrils reflect cross-bridge kinetics rather than

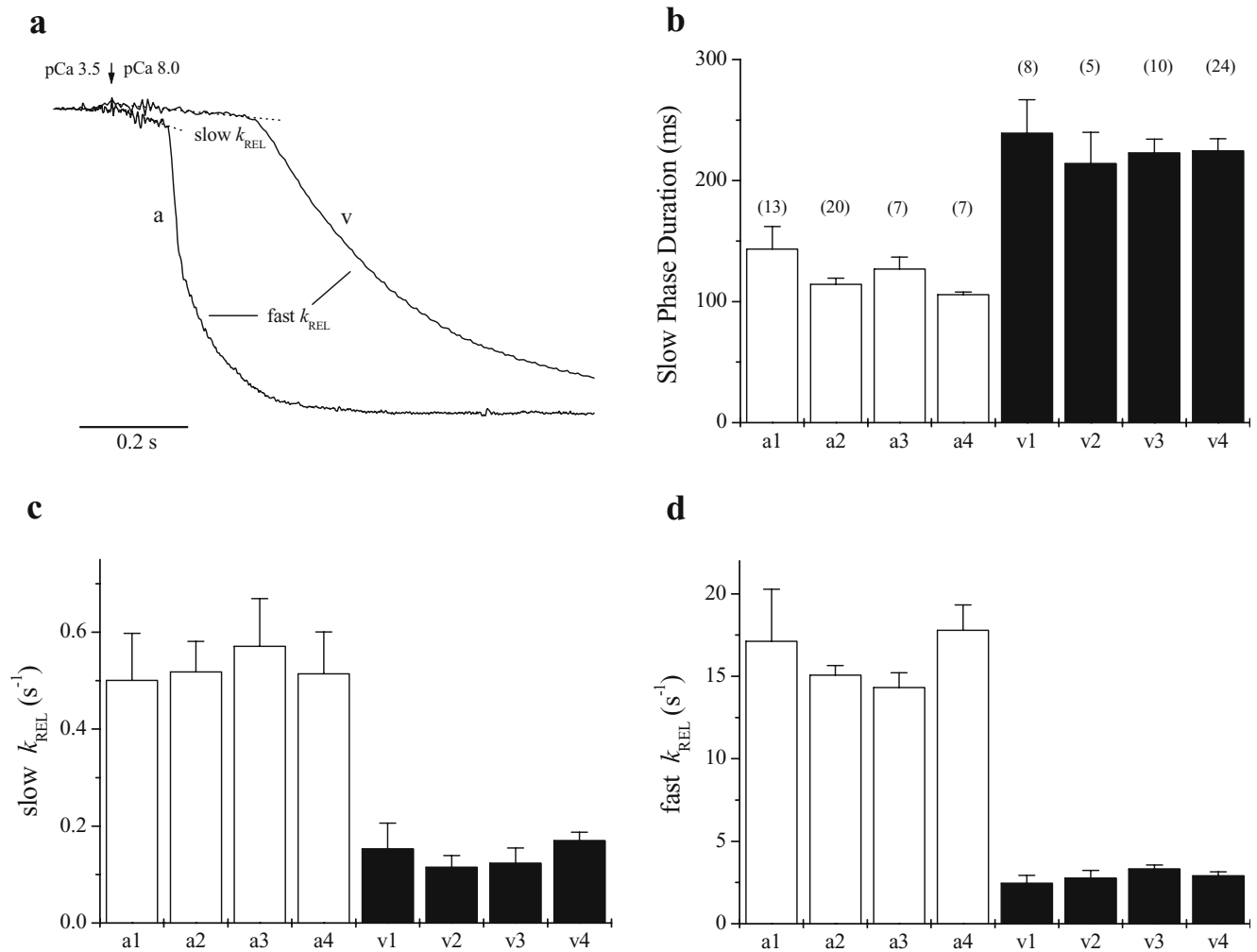


Fig. 4 Full tension relaxation initiated in human cardiac myofibrils by fast Ca^{2+} removal. **a** Representative tracings from atrial (*a*) and ventricular (*v*) myofibrils: same traces as in Fig. 2a,b superimposed on a faster time base and after normalization for maximal tension. The arrow marks the start of the solution change in the preparations. In both myofibril types full tension relaxation is biphasic. The rate constant of the early slow force decline (slow k_{REL}) is estimated from the slope of the regression line fitted to the tension trace normalized to

the entire amplitude of the tension relaxation transient. The rate constant for the final fast phase of tension decline (fast k_{REL}) is estimated from monoexponential fit. **b–d** Mean values of the relaxation parameters of the atrial (white columns) and ventricular (black columns) myofibrils obtained from each patient. Bars on top of columns are SEM; the number of myofibrils for each patient is given in parentheses in subpanel **b**

the rate at which thin filaments are switched on and off by Ca^{2+} . These kinetics seem to be consistent with simple models of regulation in which the probability of the weak to strong cross-bridge transition is modulated by $[Ca^{2+}]$.

Passive and active tension generation

The sarcomere length–resting tension relation found in ventricular myofibrils from selected patients is in excellent agreement with those previously reported for single myofibrils [21] and skinned myocardial strips [22] isolated from frozen samples of normal donor human ventricles. At rest, atrial myofibrils were significantly less stiff than the ventricular myofibrils. Considering that titin is the only

significant source of passive stiffness in single myofibrils and that two titin isoforms markedly differing in stiffness are coexpressed in cardiac sarcomeres (e.g., [16]), the result is very likely related to the lower proportion of the N2B stiff titin isoform expressed in human atrium (ca. 40% of total titin; Belus et al., unpublished results) compared to that found in human left ventricle (ca. 70%; [21, 22]).

Maximal Ca^{2+} -activated tension measured in human cardiac myofibrils ($110\text{--}130\text{ mN mm}^{-2}$) is much higher than that previously reported in mechanically isolated human cardiomyocytes ($30\text{--}50\text{ mN mm}^{-2}$ [6, 7]) and larger specimens of human myocardium ($5\text{--}20\text{ mN mm}^{-2}$ [1–5]). On the one hand, the lack of extramyofibrillar space in the cross-sectional area of isolated myofibrils and the absence

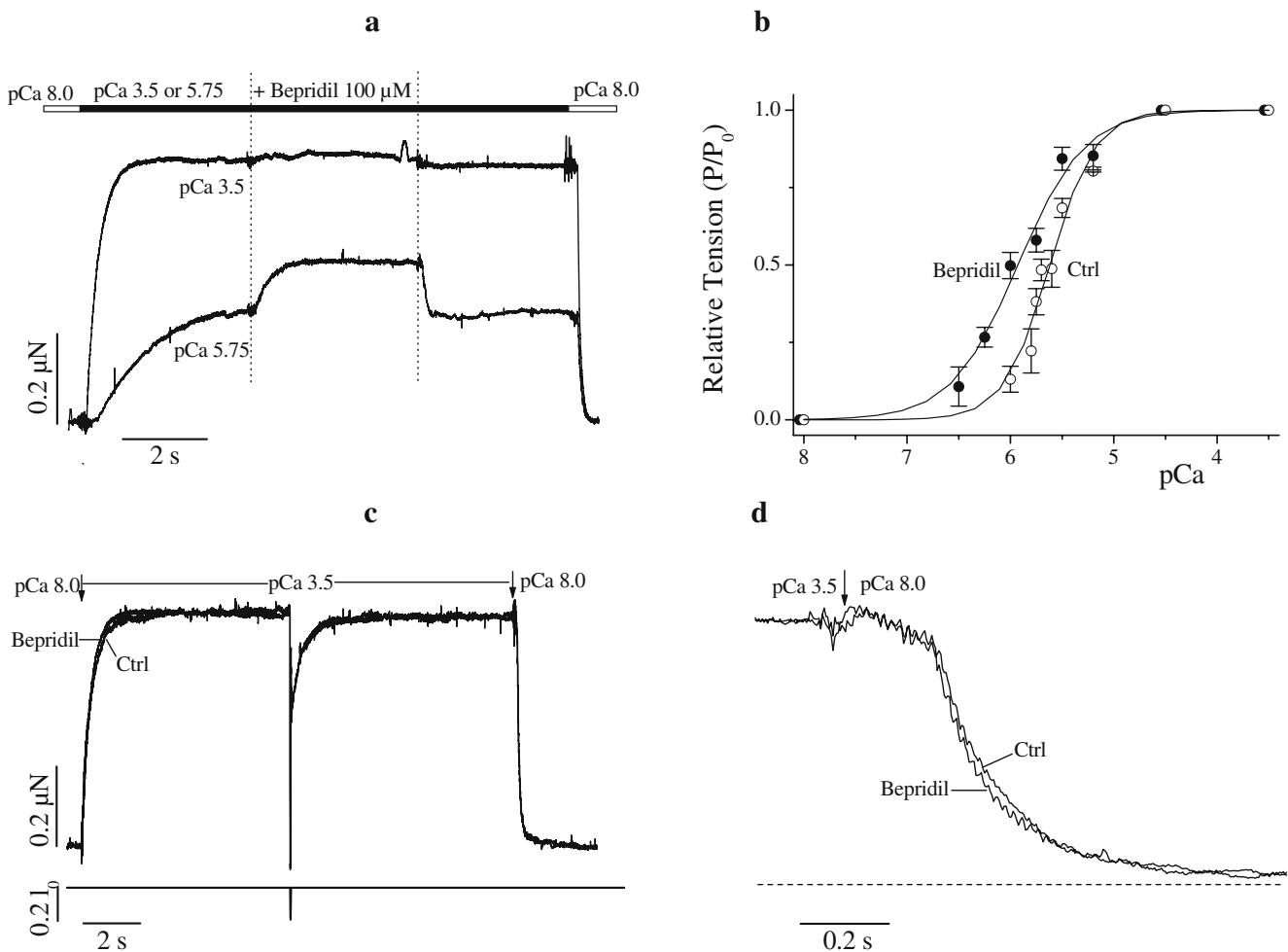


Fig. 5 Effects of Bepridil on tension and kinetics of human atrial myofibrils. **a** Maximal and submaximal tension responses of human atrial myofibril to Ca^{2+} activation by fast solution switching (pCa as indicated). Sudden exposure of the Ca^{2+} -activated myofibril to 100 μM of bepridil (between the dotted lines) causes a large, rapid, readily reversible increase of submaximal tension (pCa 5.75) but has essentially no effect on maximal tension (pCa 3.5). **b** Average pCa–tension relationships of human atrial myofibrils in the absence (closed circles, *Ctrl*) and presence (open circles, *Bepridil*) of the drug. Tension values are normalized to those measured at pCa 3.5 under each condition. Data points are means \pm SEM of three to ten myofibrils. The lines are drawn according to the parameters estimated by fitting the

data to the Hill equation; $\text{pCa}_{50} 5.63 \pm 0.04$ and 5.92 ± 0.04 , $n_{\text{H}} 2.10 \pm 0.38$ and 1.53 ± 0.23 in the absence and presence of the drug, respectively. **c** The force responses (top traces) of an atrial myofibril, maximally activated and fully relaxed by fast solution switching (pCa changes as indicated), are superimposed in the absence (*Ctrl*) and presence of 100 μM of bepridil (*Bepridil*). Fast length changes (bottom traces) are applied to the myofibril under conditions of steady tension generation to measure k_{TR} . In 11 experiments, no significant difference was found between k_{ACT} and k_{TR} values measured in the absence and presence of bepridil. **d** The time course of full tension relaxation shown in **c** is displayed on a faster time base

of P_i that depresses isometric tension (the small width of myofibril preparations prevents any significant P_i accumulation during contraction) can, at least partly, account for the difference. On the other hand, the cross-sectional area can be determined with lower precision in myofibrils than in cardiomyocytes and larger preparations of myocardium. The active tension values found in this study, in any case, agree with those reported in the best-performing cardiac preparations from animal models (e.g., [23]) and with previous reports in isolated cardiac myofibrils from mammalian and human hearts [10, 12, 24]. No significant difference was found between maximal tension developed

by atrial and ventricular myofibrils, in agreement with data showing that the higher tension of human ventricular myocardium compared to that of atrial tissue [2, 25] is mostly due to differences in myofibril density [5].

Kinetics of tension activation

Rapid elevation of $[\text{Ca}^{2+}]$ in human cardiac myofibrils by solution switching initiates tension development processes with Ca^{2+} -dependent kinetics (Fig. 3d) similar to those seen in tension transients initiated by caged Ca^{2+} photolysis or by mechanical perturbations (release–restretch) in skinned

Table 2 Means (\pm SEM) of full tension relaxation parameters in human atrial myofibrils under control conditions and after interventions that affect either troponin affinity for Ca^{2+} (bepridil 100 μM) or cross-bridge kinetics ($\text{P}_i \sim 0.2 \text{ mM}$)

Conditions	Relaxation		
	Slow phase	Fast phase	
	Duration (ms)	k_{REL} (s^{-1})	k_{REL} (s^{-1})
Control (11)	125 \pm 15	0.62 \pm 0.09	13.2 \pm 2.1
Bepridil 100 μM (11)	128 \pm 17	0.56 \pm 0.11	14.1 \pm 2.8
$\text{P}_i \sim 0.2 \text{ mM}$ (30)	88 \pm 4*	1.13 \pm 0.14*	25.2 \pm 2.5*

In the last case, the P_i mop was not added to the perfusing solutions resulting in a spectrophotometrically estimated contaminant [P_i] around 200 μM . Values in parentheses are the myofibril numbers (data from two patient samples)

All solutions contained 0.5% ethanol.

* $p < 0.01$ (vs controls) estimated by the Student t test.

striated muscle preparations from animals [18, 26]. The basis for $[\text{Ca}^{2+}]$ -dependence of k_{ACT} is an important issue because of its potential role in determining the rate of rise of force in living myocardium [26] and will be briefly discussed in the next section.

k_{ACT} measured at maximal Ca^{2+} activation in ventricular myofibrils (0.73 \pm 0.03 s^{-1} at 15°C, Table 1) is in good agreement with the rate of tension development observed after photolysis of caged ATP in human rigor left ventricular strips ($k_{\text{ATP}} = 1.53 \text{ s}^{-1}$ at 21°C; [3]) and with the rate of tension redevelopment after release–restretch in maximally activated human ventricular myocytes from donor hearts ($k_{\text{TR}} = 0.77 \pm 0.02 \text{ s}^{-1}$ at 15°C; [27]). The similarity between k_{ACT} and the apparent rates of force generation measured under conditions of full and steady thin filament activation (k_{ATP} and k_{TR}) underscores the common kinetic mechanism (the force-producing steps of the actomyosin cross-bridge cycle) that underlies all three transient responses. The idea that Ca^{2+} activation processes do not limit k_{ACT} is further strengthened by the observations that in human atrial myofibrils k_{ACT} (1) was not affected by bepridil (Fig 5c) and (2) did not increase by increasing Ca^{2+} and force levels that preceded maximal Ca^{2+} activation (data not shown).

The large difference between maximal k_{ACT} (and k_{TR}) values found in atrial and ventricular myofibrils is consistent with the functional properties of the MyHC isoforms predominantly expressed in nonfailing human atria and ventricles (80–90% α -MyHC in the atria vs 95–98% β -MyHC in the left ventricle; [17]). However, even in the slowest atrial myofibrils—that may predominantly express the β -MyHC isoform— k_{ACT} were faster than any observed k_{ACT} of ventricular myofibrils. As previously suggested [28–30], myosin light chain isoforms that are also differentially expressed in atrial and ventricular

myocardium may contribute to the large kinetic difference observed.

Kinetics of tension relaxation and Ca^{2+} regulation

Three groups of mechanisms can, in principle, be identified as the determinants of the time course of force relaxation in cardiac myocytes: (1) the rate of myoplasmic Ca^{2+} removal, (2) the kinetics of thin filament inactivation, and (3) the kinetics of cross-bridge dissociation. Rapid Ca^{2+} removal by solution switching in human cardiac myofibrils was used in this study to dissect the role of sarcomeric mechanisms from that of Ca^{2+} handling systems in the time course of force relaxation. Previous work in different myofibril types has provided evidence that cross-bridge mechanics and kinetics are major determinants of the time course of sarcomere relaxation, while thin filament inactivation kinetics do not seem to significantly limit the relaxation rate [9, 12, 15]. The present results support these conclusions.

First, the much faster relaxation kinetics of atrial vs ventricular myofibrils (Fig. 4 and Table 1) are consistent with the large difference in cross-bridge kinetics between the two myofibril types. On the other hand, the identical Ca^{2+} sensitivity of atrial and ventricular myofibrils (Fig. 3c) and the lack of major differences in the expression of thin filament regulatory protein isoforms imply that the rate at which Ca^{2+} dissociates from TnC and the dynamics of sarcomere inactivation should be similar in the two myofibril types. Second, relaxation kinetics of atrial myofibrils are not affected by bepridil (Fig. 5d and Table 2), a drug which binds to specific sites of the regulatory lobe of cardiac TnC [31] and reduces the rate at which Ca^{2+} dissociates from cardiac TnC [20]. The drug increases myofilament Ca^{2+} sensitivity (see Fig. 5b) without modifying cross-bridge properties as indicated by the lack of any effect on maximal tension, k_{ACT} , and k_{TR} of atrial myofibrils (see Fig. 5c). Third, relaxation kinetics are strongly affected by increasing [P_i] from $< 5 \mu\text{M}$ to about 200 μM , a concentration that does not affect myofilament Ca^{2+} sensitivity but is capable of increasing the rate of cross bridges detachment through reversal of the power stroke [e.g., 18].

Finally, the strong similarity between the rate constant of force relaxation during the isometric phase (slow k_{REL}) and k_{ACT} measured at low Ca^{2+} -activated force is consistent with simple two-state cross-bridge models of Ca^{2+} regulation [32, 33] in which both kinetic parameters predominantly reflect the apparent rate with which attached cross bridges leave force-generating states (g_{app}). In these models, a rapid, [Ca^{2+}]-dependent equilibration between inactive and active states of regulated actin may provide the mechanism by which the transition from weakly to strongly bound cross-bridge states becomes more likely with increasing [Ca^{2+}] due to increased

availability of actin in its active form. The increased probability that cross bridges effectively enter the force-generating states (f_{app}) increases the rate constant of force development (k_{ACT} and k_{TR}) so that the process does behave as if it is a kinetic regulation (Fig. 3d). At maximal activation, f_{app} is highest and dominates the overall isometric cross-bridge turnover ($f_{app} + g_{app}$ with $f_{app} \gg g_{app}$). During tension generation at low Ca^{2+} activation and during full relaxation after Ca^{2+} removal to subthreshold levels, the probability that cross bridges enter the force-generating states approaches zero and isometric cross-bridge turnover becomes dominated by g_{app} .

Relevance of the study

Like for other muscle types, the sarcomeric mechanisms responsible for force generation and relaxation of human cardiac muscle were dissected and directly examined in isolated myofibrils. Applying the myofibril approach to the investigation of human cardiac function can be an important step toward establishing a new method for studying cardiac sarcomeric diseases and, in perspective, a useful clinical and pharmacological assay.

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