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The lack of slow force response in failing rat myocardium: role of stretch-induced modulation of Ca–TnC kinetics

Oleg Lookin[1,2] · Yuri Protsenko[1]

Abstract

The slow force response (SFR) to stretch is an important adaptive mechanism of the heart. The SFR may result in ~ 20–30% extra force but it is substantially attenuated in heart failure. We investigated the relation of SFR magnitude with Ca^{2+} transient decay in healthy (CONT) and monocrotaline-treated rats with heart failure (MCT). Right ventricular trabeculae were stretched from 85 to 95% of optimal length and held stretched for 10 min at 30 °C and 1 Hz. Isometric twitches and Ca^{2+} transients were collected on 2, 4, 6, 8, 10 min after stretch. The changes in peak tension and Ca^{2+} transient decay characteristics during SFR were evaluated as a percentage of the value measured immediately after stretch. The amount of Ca^{2+} utilized by TnC was indirectly evaluated using the methods of Ca^{2+} transient "bump" and "difference curve." The muscles of CONT rats produced positive SFR and they showed prominent functional relation between SFR magnitude and the magnitude (amplitude, integral intensity) of Ca^{2+} transient "bump" and "difference curve." The myocardium of MCT rats showed negative SFR to stretch (force decreased in time) which was not correlated well with the characteristics of Ca^{2+} transient decay, evaluated by the methods of "bump" and "difference curve." We conclude that the intracellular mechanisms of Ca^{2+} balancing during stretch-induced slow adaptation of myocardial contractility are disrupted in failing rat myocardium. The potential significance of our findings is that the deficiency of slow force response in diseased myocardium may be diminished under augmented kinetics of Ca–TnC interaction.

Keywords Slow force response · Rat myocardium · Heart failure · Isometric contraction · Ca^{2+} transient

Introduction

The slow change in contractility after sudden stretch (slow force response, SFR) is an important regulator of pump function of the heart as this provides a long-term adaptation of the heart to the changed environmental conditions, the phenomenon known as the Anrep effect [1, 2]. This phenomenon is found in the healthy mammalian and human myocardium and observed in single cardiac cells and multicellular tissues as well as in the whole organ [3–7]. Impaired mechano-signaling to stretch in diseased myocardium, e.g., in heart failure, may lead to a lack of manifestation of SFR [8–11].

The increase in contractility (positive SFR) can be related either to extra calcium slowly accumulated in cardiac cells or slow modulation of function of intracellular molecular mechanisms involved in length-dependent activation of contraction, or both of them. The exact mechanisms remain unresolved so far, but possible pathways include activation of mechanosensitive membrane-spanning ion channels [6, 12], autocrine-paracrine regulation via the angiotensin-endothelin pathway [13, 14] involving the contribution of reactive oxygen species [15–17] and transient receptor potential canonical [18, 19], to the activation of a sodium-hydrogen exchanger, store-operated calcium channels [20, 21], and length-dependent modulation of myofilaments by the giant protein titin [22–25]. The above mechanisms can be impaired under pathological conditions, e.g., in heart

✉ Oleg Lookin
o.lookin@iip.uran.ru

1 Laboratory of Biological Motility, Institute of Immunology and Physiology, Ural Branch of Russian Academy of Sciences, 106 Pervomayskaya St., Yekaterinburg 620049, Russian Federation

2 Ural Federal University, 19 Mira St., Yekaterinburg 620002, Russian Federation

failure, and this can result in blunting or even a negative force response to sudden stretch [10]; however, there is evidence of substantial positive SFR in failing human myocardium [26]. Based on the fact that positive SFR is related to slowly increased Ca^{2+} content in cytosol/sarcoplasmic reticulum, the blunting or inversion of SFR (from positive to negative) in failing myocardium must be accompanied by contemporaneous changes in cytosolic calcium.

Our aim was to study the relation between slow force response magnitude (typically expressed as a percentage of the amplitude of the first twitch after a sudden stretch) and slow modulation of the cytosolic Ca^{2+} transient, both measured in the same muscle during the same slow response. We focused on the decay phase of the Ca^{2+} transient, not on its amplitude, as the relaxation phase of muscle contraction is of great importance in the regulation of the next beat [27, 28]. In the present study we provide new findings that relate the magnitude of SFR with the gradual slow effects on Ca^{2+} transient decay in healthy rat myocardium but not in the failing rat myocardium, where these effects were found to be substantially depressed.

Materials and methods

Ethical approval

The animals involved in the present study were cared for according to the Directive 2010/63/EU of the European Parliament and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985); their use was approved by the local institutional ethics committee. Rats were obtained from the institutional animal house and maintained under a 12-h light–dark cycle with free access to standard chow and water.

Experimental model of monocrotaline-induced heart failure

Monocrotaline was used to develop pulmonary hypertension followed by severe right ventricular hypertrophy. Briefly, 4-week-old Wistar rats of both sexes (weighing 80–100 g) were randomly divided into control (normal myocardium) and monocrotaline-treated (hypertrophic myocardium) groups. Rats in the monocrotaline-treated group were given a single subcutaneous injection of saline solution containing monocrotaline (2 ml/kg; final concentration 50 mg/kg body weight), whereas rats in the control group were injected with an equivalent volume of saline solution. Monocrotaline-treated rats (MCT, $n = 6$) were killed as soon as loss of body weight and dyspnea at rest

became prominent (~ 3.5 weeks after the treatment). Control rats (CONT, $n = 6$) were killed at the same age. The validity of the experimental model of pulmonary hypertension and severe hypertrophy/heart failure in young rats has been tested and confirmed in our recent study [29]. The above described treatment induced substantial increase in right ventricular mass with significant loss of left ventricular mass, indicating a marginal state between severe hypertrophy and heart failure (the morphometric indices relating to the heart are provided in Supplemental data, Table 1S). For uniformity, elsewhere in the text we use term "failing" to refer this state of the myocardium.

Muscle isolation and data measurements

Animals were anesthetized with 15 mg/kg zolazepam (Zoletil100®; Virbac, Carros, France) and killed by rapid cervical dislocation. The heart was quickly excised and placed in modified Krebs–Henseleit (K-H) solution (in mM: NaCl 118.5; KCl 4.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5; glucose 11.1) equilibrated in 95% O_2 + 5% CO_2 with $NaHCO_3$ and KH_2PO_4 to maintain pH at 7.35. Trabeculae were dissected from the right ventricle, clipped to a force transducer and length servomotor (Muscle Research System; Scientific Instruments GmbH, Heidelberg, Germany) in an experimental bath and continuously bathed in K-H solution. To monitor free cytosolic Ca^{2+}, the muscles were preincubated in the saline containing calcium-sensitive fluorescent indicator (5 μM fura-2/AM + 0.2% w/v Pluronic F-127) during ~ 1.5 h at room temperature and 0.2 Hz pacing rate. Prior to the measurements, the perfusion was continued in dye-free saline for 30 min. Force, length and fura-2 intensity were sampled simultaneously at 10 kHz using an analog-to-digital (A/D) and D/A data converter (PCI-1716S; AdLink Technology, New Taipei City, Taiwan) and custom-made software running in a real-time environment (HyperKernel; Nematron, Ann Arbor, MI, USA) integrated with Windows XP (Microsoft; Redmond, WA, USA). Data were processed and analyzed off-line using custom-made software. Force values were transformed to tension values using the elliptical cross-sectional area of a muscle, calculated as $S = \pi d^2/12$, where d is the wider diameter of a muscle measured at slack length. Fura-2 intensity signals were obtained as a ratio of light intensities collected at 510-nm wavelength by excitation at either 340 nm or 380 nm ($F = F340/F380$) and presented here as F/F_0, where F_0 is a ratio measured in quiescent muscle at slack length (i.e., when no stretch is applied). All experiments were carried out at 30 °C and 1 Hz pacing rate. All chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA) except potassium losartan (Merck Sharp & Dohme Corp, Haarlem, the Netherlands).

Slow force response protocol

A muscle was released to the length where no passive force was developed; this length was taken to be the slack length. The muscle was then stretched gradually for approximately 2–3% of the slack length and allowed to equilibrate at the new length prior to further measurements. When no further increase in the amplitude of active tension was observed, the final length was taken to be the optimal length (L_{MAX}). A protocol of sudden stretch of isometrically contracting muscle from 85 to 95% L_{MAX} was applied to measure slow changes in contractile activity of the muscle, i.e., slow force response (SFR), slow twitch-to-twitch changes in certain characteristics. Rapid stretch was applied during 100 ms in between two twitches and muscle force and Ca^{2+} transients at the new length were recorded for ~30 s around the time intervals of 2, 4, 6, 8 and 10 min after the stretch (see Fig. 3). To compare the expression of slow changes in contractility between individual muscles, we normalized the value of given characteristics in each twitch to the value obtained from the first twitch after stretch.

The analysis of Ca^{2+} transient decay

We focused our analysis on the decay phase of Ca^{2+} transient rather than Ca^{2+} transient amplitude (see Discussion for reason). We applied two distinct methods to reveal whether slow force response to stretch is related to slow (if any) changes in the decay of the Ca^{2+} transient: the method of "bump" (the first observation and further concept were introduced two decades ago [3] and described in detail elsewhere [30]) and the method of "difference curve."

It has been shown previously [29, 30] that there is a brief delay in Ca^{2+} transient decay ("bump") in stretched rat myocardium, compared to non-stretched muscle (Fig. 1a). The consistent effect of gradual muscle stretch is an increase in the magnitude of the "bump" (Fig. 1b). We evaluated the characteristics of the "bump" — amplitude, integral intensity, time-to-peak — during the development of the slow force response, according to our method [30, 31]. The amplitude of the "bump" was expressed as a percentage of the Ca^{2+} transient amplitude (normalized "bump" amplitude), the integral intensity of the "bump" was expressed as a percentage of the area calculated under the monotonic part of the Ca^{2+} transient, and the time-to-peak of the "bump", i.e.,
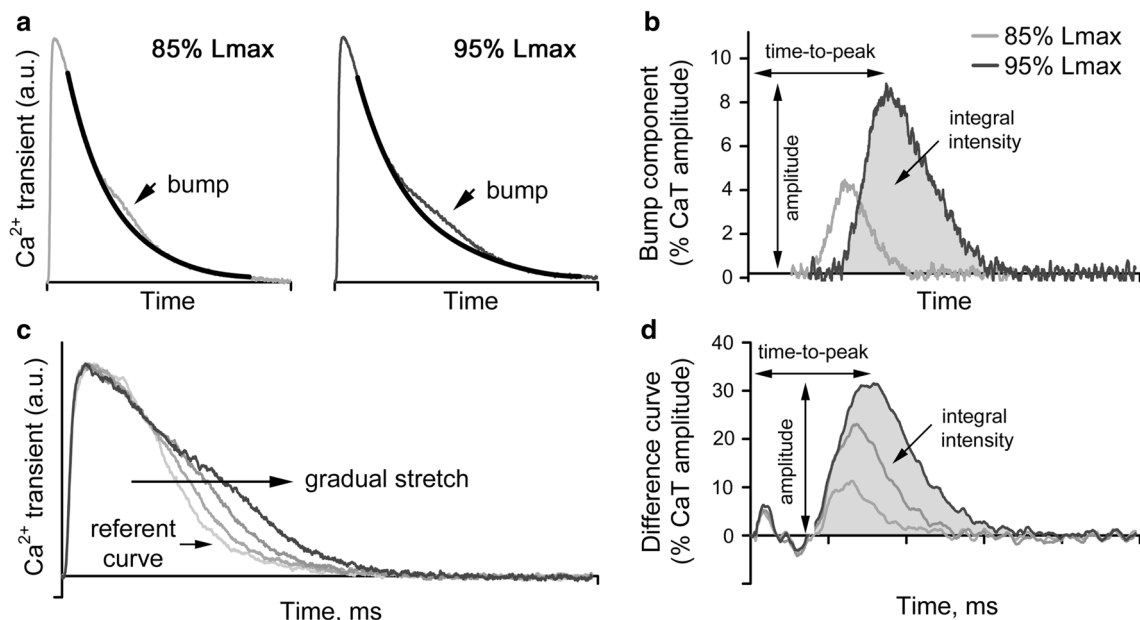

Fig. 1 The methods of "bump" and "difference curve" of Ca^{2+} transients. **a** Ca^{2+} transient traces obtained under low or high stretch of a muscle (e.g., 85% and 95% L_{MAX}) and normalized to their peaks. *Thin line* shows raw Ca^{2+} transient trace, *thick line* shows its monotonic component. The difference between the lines ("bump") is indicated by *arrows*. **b** The time-based "bump" traces obtained from the transients shown in panel **a**. Certain characteristics of the "bump" are used for the analysis, e.g., its amplitude, integral intensity (an area under the "bump" trace), time-to-peak. **c** The superposition of normalized Ca^{2+} transient traces obtained under gradual stretch of a muscle as indicated by a *long arrow*. The trace obtained under no or minimal stretch is assumed as referent curve and this curve is subtracted from any other curve in the set. **d** The superposition of time-based "difference curves" obtained from the transients shown in panel **c**. Like the "bump", the "difference curve" has certain characteristics, e.g., amplitude or integral intensity. Note that the difference curve has at least two components which are deflected upward ("positive" component) and downward ("negative" component). The characteristics shown here in panel **d** are related to the "positive" component of the "difference curve." Please see the text for a detailed description

the time of maximal deflection of bumped part of the Ca^{2+} transient trace from its monotonic component, was evaluated relative to the Ca^{2+} transient time-to-peak.

We also developed another approach ("difference curve") in which we evaluated the difference between two Ca^{2+} transient traces obtained for two different conditions, e.g., for different time intervals during the slow force response. Prior to the calculation of "difference curve", Ca^{2+} transient traces were normalized to their respective peaks. As shown in Fig. 1c, a gradual stretch of rat cardiac muscle modifies the Ca^{2+} transient decay phase in a very distinct manner. If some condition is used as the referent, e.g., where lower stretch of the muscle is applied, then the corresponding Ca^{2+} transient trace can be subtracted from the trace obtained for another condition. Figure 1d demonstrates the series of "difference curves" retrieved from the Ca^{2+} transient traces obtained under gradual stretch of a muscle, shown in Fig. 1c. Like the "bump" curve, the "difference curve" can be characterized by certain features, including amplitude, integral intensity and time-to-peak of the curve. A common feature of the "difference curve" is that it has at least two distinct phases or components: one is being deflected upward ("positive" component, its characteristics are indicated in Fig. 1d) and another is being deflected downward ("negative" component). In the present study we focused on the "positive" component of the "difference curve" (referred to in figure legends as DC), which is larger and slower (see Discussion for reasons). It was evaluated by its amplitude, integral intensity (an area under this component of the difference curve) and time-to-peak (calculated from the time-to-peak of Ca^{2+} transient). Also, as we used the Ca^{2+} transient measured immediately after stretch as the referent transient, we analyzed slow changes in the "difference curve" in a distinct manner, compared to the analysis of the "bump." At the time point of 0 min after stretch (immediately after stretch), no "difference curve" exists. Therefore, the amplitude and integral intensity of the "difference curve" are presented as a percentage of Ca^{2+} transient amplitude/integral intensity, not as a percentage of the first value after the stretch. The possible consequences of using the "difference curve" method specifically to analyze Ca^{2+} transients are discussed in detail in the appropriate section.

Statistical analysis

The number of preparations involved in the analysis is equal to the number of animals used and indicated in figure legends as "*n*." The time-matched differences in peak tension and the characteristics of the Ca^{2+} transient decay phase between normal and failing myocardium during the development of slow force response (at 2 min after stretch, at 4 min after stretch, and so on) were evaluated by a Mann–Whitney *U* test. The time-dependent effects in the same group, e.g.,

the comparison of the means for the 2-min vs 10-min time point, were tested by a Wilcoxon matched pairs test. Effects were considered significant at $P < 0.05$. Data are presented either as median with 25–75% percentiles or as mean $\pm$ SD. The statistical analysis was performed with Statistica 8.0 (StatSoft Inc.).

Results

The magnitude of slow force response in normal and failing rat myocardium

The magnitude of slow force response is presented here as a percentage of the amplitude of first twitch after sudden stretch. Figure 2 summarizes slow force responses measured in the muscles from CONT and MCT rats. Sudden stretch is accompanied by a gradual time-dependent increase in active tension in CONT rats, while MCT rats showed a negative response (Fig. 2). During the progression of slow response in contractility from the 2-min to the 10-min time point, the mean value of SFR magnitude in the CONT group increased from $105.9 \pm 2.5\%$ to $110.9 \pm 6.0\%$ of the first value after stretch (mean $\pm$ SD, significant difference, $P = 0.046$ by Wilcoxon matched pairs test). In the group of monocrotaline-treated animals, the corresponding mean values of SFR magnitude were $93.5 \pm 5.0\%$ and $93.7 \pm 6.3\%$ (mean $\pm$ SD, non-significant difference, $P = 0.92$, Wilcoxon matched pairs test). At the end of the 10-min interval, the muscles from normal and failing rat hearts were significantly different in the SFR magnitude ($110.9 \pm 6.0\%$ in CONT vs $93.7 \pm 6.3\%$ in MCT, mean $\pm$ SD, $P = 0.004$, Mann–Whitney *U* test). Therefore, the failing rat myocardium was substantially different from the healthy hearts in the response to sudden mechanical stretch.

Slow changes in "bump" characteristics in response to stretch

In order to evaluate how the slow force response is related to (if any) slow changes in Ca^{2+} transient decay phase, we measured a series of Ca^{2+} transients around the time intervals of 2, 4, 6, 8 and 10 min after the sudden stretch was applied (Fig. 3) and analyzed the changes using the "bump" and "difference curve" methods separately. Please note that we used normalized Ca^{2+} transients, according to the reasons described in "Materials and methods."

The time-based changes in the normalized amplitude, integral intensity and time-to-peak of the Ca^{2+} transient "bump" measured in healthy and failing rat myocardium are shown in Fig. 4. The magnitude of the "bump", as assessed by its amplitude and integral intensity, was progressively elevated in CONT muscles during the SFR,
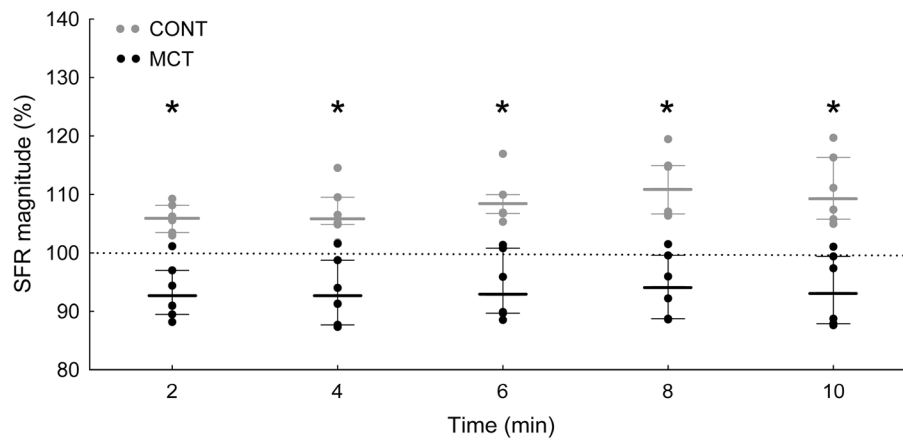
Fig. 2 The slow force response to sudden stretch in normal and failing rat myocardium. The slow change in isometric tension in response to sudden stretch in normal ($n = 6$) and failing ($n = 6$) rat myocardium. SFR magnitude is expressed as a percentage of the amplitude of first twitch after stretch. The *dotted line* shows the level of "no change."

Scatter-plots of raw data (*dots*), median values (*long thick lines*) and 25–75% percentiles (*short thin lines*) are shown. *Asterisks* indicate that the mean values are significantly different between CONT and MCT groups for the given time (Mann–Whitney U test, $P < 0.05$)

Fig. 3 The scheme demonstrating the simultaneous measurement of slow force response and Ca^{2+} transients at the preset time intervals of SFR. The *top* of the diagram shows typical Ca^{2+} transients in the muscle of CONT rat; the *bottom* part shows typical Ca^{2+} transients in the muscle of MCT rat. The *center* of the plot shows typical SFR measured in the muscles from CONT and MCT rats. *Arrows* indicate time intervals where Ca^{2+} transients were collected
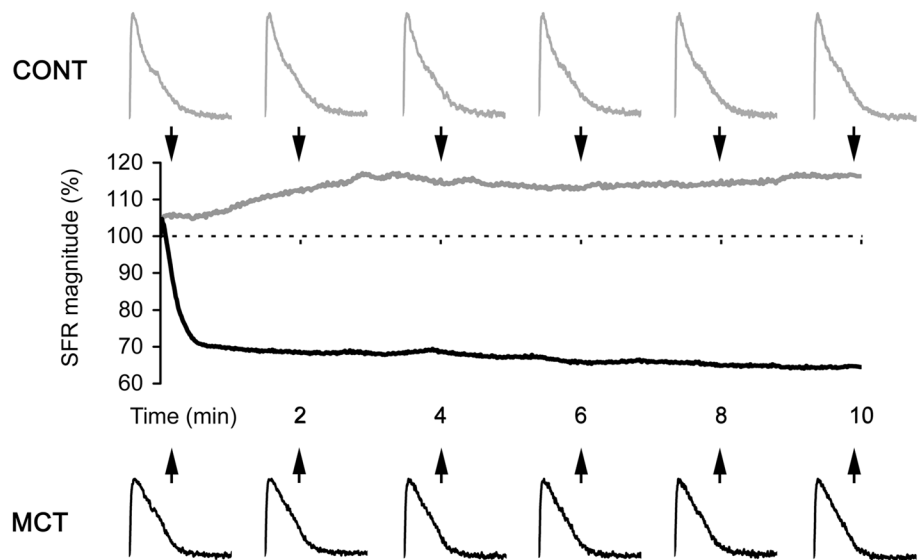
while these "bump" characteristics in MCT muscles slowly decreased over time (Fig. 4a, b). At the end of the response (at 10 min after stretch), the mean values of "bump" amplitude amounted to $120.2 \pm 6.9\%$ and to $74.2 \pm 14.3\%$ of the first value after stretch in healthy myocardium and failing myocardium of monocrotaline-treated rats, respectively (mean $\pm$ SD, $P = 0.0039$, Mann–Whitney U test). The mean values of "bump" integral intensity at the same stage of SFR (at the end of 10 min) amounted to $139.4 \pm 11.2\%$ and to $72.7 \pm 14.0\%$ of the first value after stretch in CONT and MCT groups, respectively (mean $\pm$ SD, $P = 0.0039$, Mann–Whitney U test). A much less regular change in "bump" time-to-peak was found

both in CONT and MCT groups (Fig. 4c) and this parameter showed non-significant elevation during SFR in both groups ($P = 0.116$ and $P = 0.075$ for CONT and MCT, respectively, Wilcoxon matched pairs test). Also, at the end of a 10-min interval of sustained stretch, the values of "bump" time-to-peak in CONT and MCT groups were, respectively, $106.8 \pm 11.1\%$ and $108.8 \pm 20.0\%$ of the first value after stretch (mean $\pm$ SD, non-significant difference, $P = 1.0$, Mann–Whitney U test). These findings indicate that the amount, rather than the timing properties, of the Ca–TnC kinetics plays a role in the expression of SFR in rat cardiac muscle.
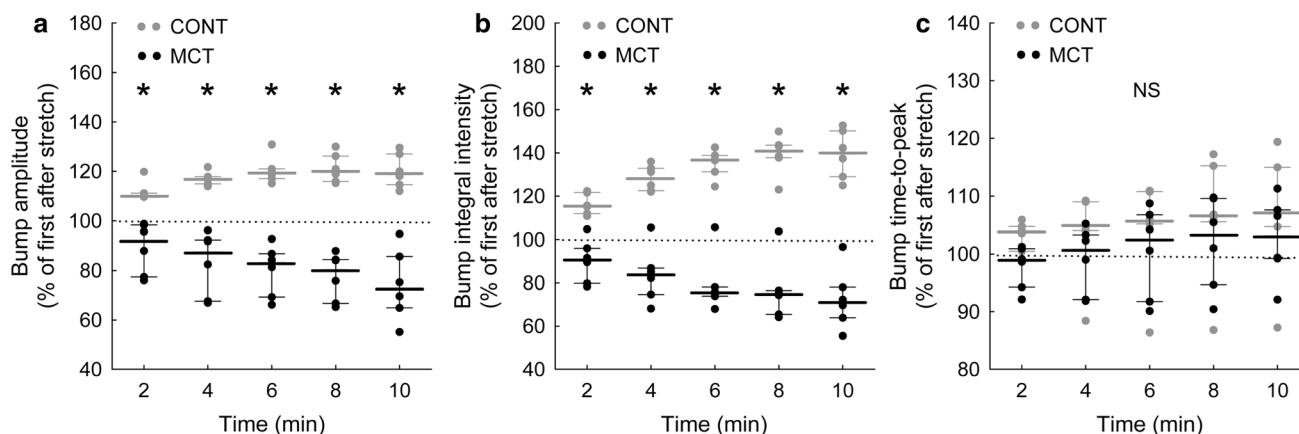
Fig. 4 The time-based changes of the Ca^{2+} transient "bump" characteristics during slow force response to sudden stretch. The changes in Ca^{2+} transient "bump" characteristics during the development of slow force response in normal ($n=6$) and failing ($n=6$) rat myocardium. **a** "Bump" amplitude. **b** "Bump" integral intensity. **c** "Bump" time-to-peak. All the characteristics are presented as a percentage of the first value after stretch. Scatter-plots of raw data (*dots*), median value (*long thick lines*) and 25–75% percentiles (*short thin lines*) are shown. The *dotted line* shows the level of "no change." *Asterisks* indicate that the mean values are significantly different between CONT and MCT groups for the given time (Mann–Whitney U test, $P < 0.05$). *NS* no significant difference

Slow changes in "difference curve" characteristics in response to stretch

Figure 5 summarizes time-based changes in the amplitude, integral intensity and time-to-peak of the "positive" component of "difference curve", obtained by subtraction of the Ca^{2+} transient measured immediately after stretch from the Ca^{2+} transients measured at 2, 4, 6, 8 or 10 min after the stretch, for healthy and failing rat myocardium. Note that

these characteristics are presented in actual values, not as a percentage of the first value after stretch. This is due to the feature of the method of the "difference curve" where Ca^{2+} transient immediately after stretch (0 min after stretch) was used as a referent.

At the early stage of sustained stretch, there is an increase in the amplitude and integral intensity of the "positive" component of the "difference curve" both in healthy and failing rat myocardium (Fig. 5a, b). However,
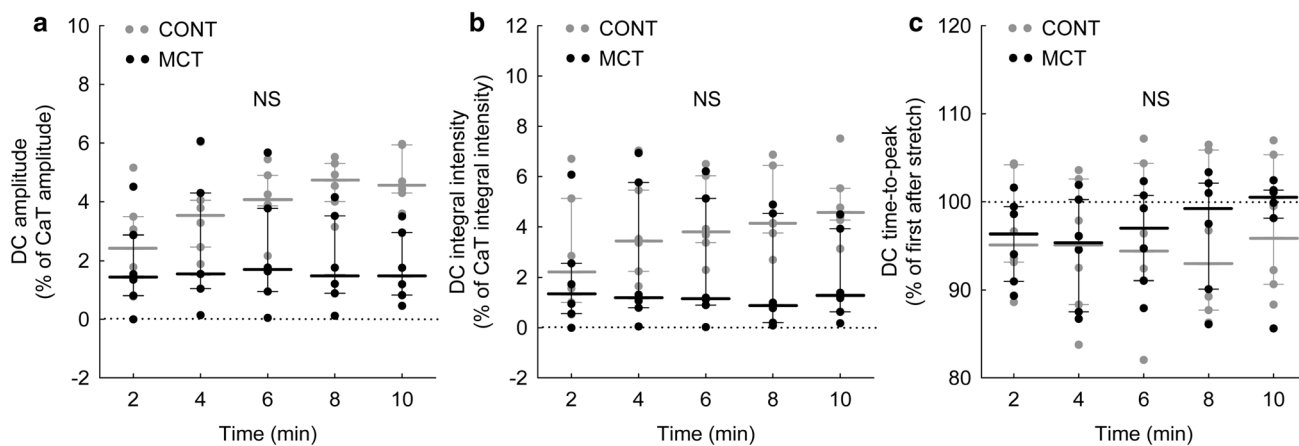

Fig. 5 The time-based changes of characteristics of the "difference curve" ("positive" component) during slow force response to sudden stretch. The changes in the characteristics of the "positive" component of the "difference curve", obtained by subtraction of referent Ca^{2+} transient from a current one, during the development of slow force response in normal ($n=6$) and failing ($n=6$) rat myocardium. **a** Amplitude of the "positive" component of the "difference curve." **b** Integral intensity of the "positive" component of the "difference curve." **c** Time-to-peak of the "positive" component of the "difference curve." *DC*, "difference curve"; *CaT*, Ca^{2+} transient. Note that the characteristics are presented in actual values, not as a percentage of the first value after stretch. Scatter-plots of raw data (*dots*), median values (*long thick lines*) and 25–75% percentiles (*short thin lines*) are shown. The *dotted line* shows the level of "no change." *NS*, no significant difference was found between CONT and MCT groups for the given time interval (Mann–Whitney U test)

only the CONT group displayed slow additional elevation of the values of these parameters during SFR whereas those in the MCT group remained virtually unchanged. For instance, the amplitude of the "positive" component of the "difference curve" in the CONT group increased from $2.7 \pm 1.5\%$ of Ca^{2+} transient amplitude at the 2-min point to $4.8 \pm 0.9\%$ of Ca^{2+} transient amplitude at the 10-min point (mean $\pm$ SD, significant difference, $P = 0.027$, Wilcoxon matched pairs test). In contrast, in the MCT group this parameter was $1.9 \pm 1.6\%$ and $1.8 \pm 1.2\%$ of Ca^{2+} transient amplitude at the 2-min and 10-min time points after the stretch (mean $\pm$ SD, non-significant difference, $P = 0.753$, Wilcoxon matched pairs test). The integral intensity of the "difference curve" increased from $3.0 \pm 2.4\%$ to $4.9 \pm 1.5\%$ of Ca^{2+} transient integral intensity (the area under the Ca^{2+} transient curve) at the 2-min time point vs the 10-min time point, respectively; the change was significant ($P = 0.028$, Wilcoxon matched pairs test). The MCT group showed no significant time-dependent variation of the value of this parameter: $2.0 \pm 2.2\%$ and $2.0 \pm 1.8\%$ of Ca^{2+} transient integral intensity at 2-min and 10-min time points, respectively ($P = 0.92$, Wilcoxon matched pairs test). On the other hand, no significant difference was observed between the mean values of CONT vs MCT group collected for any given time interval (Fig. 5a, b). Similarly, the mean values of "bump" time-to-peak were not significantly affected by sudden stretch in either group, and were insignificant between the two groups regardless of the time interval (Fig. 5c).

The correlation between SFR and slow changes in the characteristics of Ca^{2+} transient decay

Due to the high variability of the calculated data (amplitude, integral intensity etc., see Fig. 5) it is uncertain how the slow changes in the characteristics of the Ca^{2+} transient relate to the magnitude of slow force response. To provide detailed insight to the possible mutual changes in peak isometric tension and the properties of Ca^{2+} transient decay, we analyzed the characteristics of Ca^{2+} transient decay (the amplitude and integral intensity of the "bump" and the "positive" component of the "difference curve") being plotted against SFR magnitude in a time-matched manner (Fig. 6). In healthy rat myocardium, we found a high correlation between slow increase in active tension and slow increase in these characteristics of the "bump" and "difference curve." For example, the determination of the coefficients (R^2 values) of linear approximation used for the relationships of the CONT group, which are presented in Fig. 6, were all between 0.94 and 0.99. This visual representation also revealed that the linear approximation for the averaged data obtained from healthy myocardium, if extended to the interception of the two axes, comes near to the "no change" point. In other words, if SFR magnitude is $\sim 100\%$, i.e., no or negligible slow changes in active tension detected during SFR, then the "bump" or "difference curve" characteristics show minimal change relative to the value in the first twitch after stretch.

In contrast, there was a lack of regularity between slow changes in active tension and slow changes in the
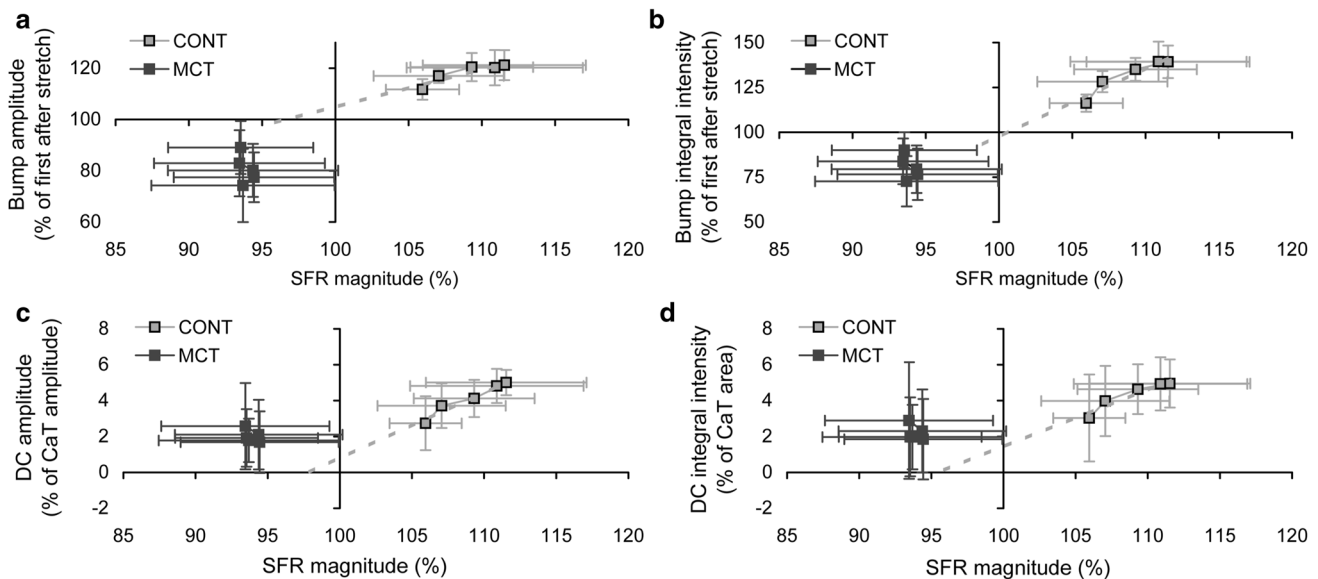

Fig. 6 The relations between time-matched SFR magnitude and the slow changes in the characteristics of the Ca^{2+} transient "bump" or "difference curve." **a** "Bump" amplitude is plotted against SFR magnitude. **b** "Bump" integral intensity is plotted against SFR magnitude. **c** The amplitude of the "positive" component of the "difference curve" is plotted against SFR magnitude. **d** The integral intensity of the "positive" component of the "difference curve" is plotted against SFR magnitude. *DC*, "difference curve"; *CaT*, Ca^{2+} transient. Data is shown as mean $\pm$ SD. *Dashed lines* show linear approximation for CONT data

characteristics of "bump" or "difference curve" of the Ca^{2+} transient. While the "bump" amplitude and "bump" integral intensity, if plotted against SFR magnitude, did show quite high R^2 values of linear approximation (0.7 and 0.82, respectively, Fig. 6a, b), the actual change of these characteristics had no reasonable dependence on SFR magnitude, since the mean value points were spread in a very dense space on the X axis (~2% of SFR magnitude) and progressed in parallel to the Y axis only. The amplitude and integral intensity of the "positive" component of the "difference curve" were totally uncoupled with slow changes in active tension, since the linear approximations of the mean data relationships showed R^2 values of 0.07 and 0.21, respectively (Fig. 6c, d). The result indicates that the stretch does modify Ca^{2+} transient decay in both healthy and failing myocardia; however, no contemporary slow changes in Ca^{2+} transient decay are presented in the failing rat heart, compared to normal hearts. The interpretation of the finding is that the slow modulation of Ca–TnC kinetics, typical for healthy cardiac muscle, and possibly playing a key role in the slow force response, is substantially impaired or has even vanished in the failing myocardium.

In the final step of our study, we compared the calculated dependences obtained by use of "bump" and "difference curve" of Ca^{2+} transient decay, in order to reveal whether the methods are interchangeable. Since we obtained a reasonable correlation between the mechanics and Ca transient decay only for the control group of muscles, we compared those sets of data. We plotted the above stated dependences (amplitude and integral intensity) of the "bump" and the "positive" component of the "difference curve" against SFR magnitude and superimposed them (Fig. 7). Importantly, we found a good degree of similarity between the methods in regards to calculated relationships, in spite of the methods having principally different initial steps of analysis. As shown in Fig. 7a, c, there is some dissimilarity between the calculation of the amplitudes of the "bump" and the "positive" component of the "difference curve", so the plot does not follow a straight line. In contrast, the relationships between SFR magnitude and the integral intensities of the "bump" and the "positive" component of the "difference curve" showed very high coincidence (Fig. 7b). If integral intensities of the "bump" and the "positive" component of the "difference curve" are plotted against each other, this relation was found to be quasilinear (Fig. 7d). Accordingly, we can confirm the applicability of the method of the "difference curve" to reveal changes in the Ca^{2+} transient decay phase, similarly to the previously used method of "bump" analysis.
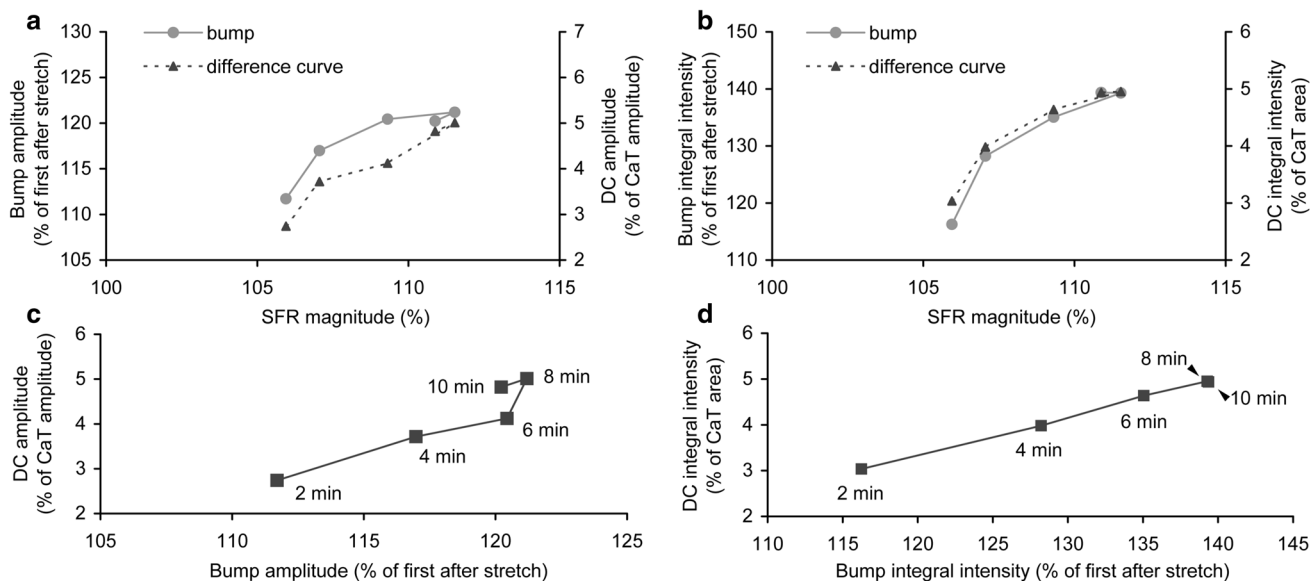

Fig. 7 The comparison of calculation similarities between the methods of "bump" and "difference curve" used to characterize Ca^{2+} transient decay during slow force response to sudden stretch (in CONT group only). **a** The amplitudes of "bump" and the "positive" component of the "difference curve" are plotted against SFR magnitude. **b** The integral intensities of "bump" and the positive "component" of the "difference curve" are plotted against SFR magnitude. Note use of secondary axis in panels **a** and **b**. **c** Amplitudes of "bump" and "positive" component of the "difference curve" are plotted against each other. **d** Integral intensities of "bump" and "positive" component of the "difference curve" are plotted against each other. *DC*, "difference curve"; *CaT*, Ca^{2+} transient. Mean values are shown. Labels on panels **c** and **d** show time interval after stretch which corresponds to the nearest point. *Arrows* on panel **d** show the exact position of the point for time intervals labeled "8 min" and "10 min"

Discussion

For the first time we obtained evidence of interrelation between the magnitude of slow force increase and slow changes in Ca^{2+} transient decay in healthy rat cardiac muscles subjected to sudden stretch. This interrelation could indicate that there is a direct interplay between slowly developed modulation of Ca–TnC kinetics and additional activation of muscle contraction. The results obtained show that the percentage increase in peak active tension during slow response is substantially lower than that of certain characteristics of the Ca^{2+} transient "bump." For example, in our set of muscles from healthy hearts the maximum SFT magnitude was ~ 10% over the force at first twitch after stretch, while the "bump" integral intensity achieved ~ 40% over the first value after stretch at the end of response. Also, the time-matched plots of SFR magnitude vs "bump" or "difference curve" amplitude/integral intensity showed saturation-like behavior, i.e., with large changes at the beginning of the response but minor changes at the end of the response (see Fig. 6). This may indicate that there is a "gain coefficient" between slow modulation of Ca–TnC kinetics and additional activation of contraction.

In failing rat myocardium, we observed a blunted or even negative slow force response to sudden stretch, as has been reported previously [8, 9, 29, 31]. This deficiency in SFR magnitude was accompanied by the deficiency in slow changes in Ca^{2+} transient decay. Moreover, the contemporary changes in peak active force and Ca^{2+} transient decay during the progress of the slow response were significantly depressed so the interrelation between them was low or negligible. Indeed, the linear approximation between SFR magnitude and certain characteristics of Ca^{2+} transient decay, retrieved on the basis of the methods of "bump" and "difference curve", was poor enough so as to have determination coefficients $R^2 < 0.1$.

Combined together, these findings demonstrate the potential role of the kinetics of Ca^{2+} extrusion from the cytosol, as monitored by Ca^{2+} transients, in the expression of slow force response. Yet, the slow drift of Ca^{2+} transient decay properties may reflect the slowly progressing changes in the kinetics of Ca-utilizing mechanisms following thesudden change in muscle length. Ca^{2+} buffering by troponin C is one of the potent mechanisms contributing to the magnitude of SFR and this buffer capacity depends both on sarcomere length and the function of the cardiac cell. It is known that failing myocardium has decreased length-dependence of Ca^{2+} sensitivity of myofilaments which accounts for the blunted Frank-Starling mechanism and may contribute to a depressed slow force response [8, 9, 32]. However, the length-induced effects on myofilament activation seem not to be the only mechanism for slow modulation of contraction, because positive SFR (while lower than in a normal heart) was observed in human failing ventricular and atrial myocardium [7, 9, 26]. The impaired function of mitochondria in failing hearts results in lowered ATP content available for recycling of myosin cross-bridges and this, via cooperative activation of myofilaments, affects the total amount of Ca–TnC complexes activated during the contraction cycle [32, 33]. Importantly, the kinetic properties of association and dissociation of Ca^{2+} and TnC strongly modulate relaxation of cardiac muscle, the Ca^{2+} content in intracellular Ca^{2+} stores and the contractile ability of cardiomyocytes in the following beat cycle(s) [34, 35]. For instance, the elevated amount of Ca^{2+} utilized by TnC and/or total prolongation of Ca–TnC kinetics affects the Ca^{2+} load in sarcoplasmic reticulum (SR) via modulation of SERCA2a function, and this may result in a slow increase in Ca^{2+} transient amplitude [3, 36, 37]. Indeed, it has been shown that Ca^{2+} transient amplitude is not rapidly increased immediately after the sudden stretch but progressively elevated during the slow force response [3, 38, 39]. Moreover, it has been reported that stretch-induced elevation of systolic pressure in a whole heart preparation does not relate to the changes in myoplasmic Ca^{2+} [40]. Impaired function of SERCA2a in failing heart may distort the process of slow balancing of Ca^{2+} between cytosol and SR, including the contribution of a sodium-calcium exchanger (NCX) into this complex stabilization [35, 41–45]. The metabolic dysfunction, e.g., the depression of mitochondrial function by reduction of the content or activity of creatine kinase, is a key factor of such impairment of calcium homeostasis [46–48], while mitochondrial Ca^{2+} uptake plays a small role in the direct regulation of cytosolic calcium [28]. Also, a decrease in Ca^{2+} sensitization of myofilaments in the failing heart may contribute to the deficiency in positive SFR to stretch, similar to the negative force-frequency response in the failing myocardium [49, 50]. Taken together, it may be concluded that the fine regulation of beat-to-beat increase in contractility after sudden stretch might be related to the slow Ca^{2+} redistribution between (intra)cellular compartments, arising predominantly from the beat-to-beat balancing between Ca–TnC kinetics and SERCA2a function [34, 51]. Our results, obtained in failing rat myocardium, confirm this concept, because we observed here both negative SFR and the decrease in the magnitude of the Ca^{2+} transient "bump" and "difference curve." Yet, the most plausible situation is the combined effect of numerous mechanisms which affect Ca^{2+} transport across the cell membrane and/or between intracellular Ca^{2+} stores (e.g., stretch-activated channels, store-operated calcium channels, NCX, change in intracellular pH etc., [38, 39, 52]) and the modulation of the intracellular Ca^{2+} cycle by the kinetics of Ca–TnC.

The reason we say this, based on the present data, is the lack of prominent interrelation between the mechanics and free cytosolic Ca^{2+} kinetics in failing myocardium, where poor correlation was found between them. In the case of the ultimate role of Ca–TnC kinetics in the slow force response, we must find the complete inversion of the responses (from positive to negative) in the failing heart, but minor distortion of the functional relation between the mechanics and Ca^{2+} transient decay must exist. On the other hand, in the failing rat myocardium we observed virtually no progression of slow change in active tension during SFR, just the immediate deficiency after the stretch. Therefore, the slow changes in Ca^{2+} transient decay may be too little to be adequately detected in the response, and this severely affects the observed relation between SFR magnitude and certain characteristics of Ca^{2+} transient decay.

In the present study we introduced our new method of "difference curve" designed specifically to analyze the Ca^{2+} transient decay phase in order to reveal whether the slow force changes are related to the changes in this decay. In our recent studies we found a lack of the length-dependent change in "bump" amplitude and integral intensity of Ca^{2+} transients measured in the right ventricular trabeculae of monocrotaline-treated rats with prominent signs of heart failure [29–31]. In fact, we observed the deficiency in the "bump" itself in those preparations, but their Ca^{2+} transients still had substantial and progressive change in the decay phase, which were in parallel with gradual stretch. Therefore, we needed to develop an alternative approach to investigate how stretch of the muscle modulates its Ca^{2+} transient. Dissimilar to the method of "bump" analysis where each Ca^{2+} transient is inspected independently for the monotonic decay and non-monotonic "bump" [30], the method of "difference curve" is related to the difference between a referent Ca^{2+} transient (assumed to be the state before the effect) and each consecutive Ca^{2+} transient (the progression of the effect). Also, the principal difference between the "bump" and "difference curve" analysis is that the former is just a part of the Ca^{2+} transient decay while the latter follows Ca^{2+} transient decay, on the whole. This, therefore, makes it possible to study the changes in free cytosolic calcium kinetics occurring in a very early or late phase of Ca^{2+} transient decay.

The typical "difference curve" has three components (all can be seen in Fig. 1d). The first upward component occurs within 20–30 ms from the stimulus, indicating that there is some stretch-related effect on calcium-induced calcium release; it virtually coincides with the peak of the Ca^{2+} transient and therefore is not related to the length-induced changes in Ca^{2+} utilization by troponin C. The exact mechanism for this component is unknown but it may occur due to additional Ca^{2+} entering the cell via NCX or stretch-activated channels. The "negative" downward component starts to develop nearly around the Ca^{2+} transient peak and lasts several tens of milliseconds. Probably, it corresponds to the fast Ca^{2+}-utilization by TnC in stretched muscle, giving the decreased free cytosolic Ca^{2+} compared with the non-stretched muscle. However, this component was found to be not very dependent on the stretch of the muscle, compared to the third "positive" upward component which is greatly influenced by stretch of the muscle, both in regards to amplitude and timing properties. For example, under gradual stretch (e.g., in force–length relation) this component shows a large increase in amplitude and integral intensity accompanied by substantial deceleration of its development, i.e., by an increase in time-to-peak. All these length-induced changes indicate that this "positive" component may be highly related to the kinetics of Ca–TnC interaction which is augmented and prolonged in stretched muscle, compared to the non-stretched state. Therefore, in our manuscript we have focused on this component of the "difference curve" only, while omitting the first two components from the analysis.

The method of the "difference curve" does not require special algorithms to find any specific part of the Ca^{2+} transient decay, unlike the "bump" method of analysis, though some calculations are needed to distinguish between "negative" and "positive" components (see Fig. 1d). Finally, and importantly, in the opinion of the authors, the method of "difference curve" is invariant to the occurrence of the specific "bump" on the Ca^{2+} transient decay, is applicable to transients of arbitrary shape, and therefore can follow prolonged and/or small changes in the kinetics of Ca^{2+} transient decay. Altogether, we conclude that the use of the "difference curve" instead of the "bump" is preferred in order to evaluate precisely the effects on Ca^{2+} transient decay during the relaxation phase of cardiac muscle.

The study has some methodological limitations. For instance, the measurements were done at a temperature and pacing rate substantially lower than the physiological conditions in rat myocardium. We were limited to these conditions since the Ca-sensitive fluorophore fura-2 was subjected to rapid gradual photodeactivation at physiological temperature/pacing rate while the SFR measurements lasted tens of minutes. Also, in contrast to the previously reported slow rise in Ca^{2+} transient amplitude during slow force response [3, 12, 18], we did not observe such changes except a few examples (please see Fig. 1S in Supplemental data). It may be that cumulative photodegradation of fluorescent dye during long recordings lasting several tens of minutes (both due to the effect of periodical illumination, bleaching of the dye, and a temperature-related effect) contributes to the lack of increase in the Ca^{2+} transient amplitude. Another reason for discrepancies between our results and results provided by others may be the use of different fluorescent dyes (e.g., with

different affinity to Ca^{2+}) and/or different loading times. On the other hand, we focused on more precise assessment of the decay phase of the Ca^{2+} transient rather than its amplitude, as it has been agreed that the relaxation of cardiac muscle is a key step of the cardiac cycle and it needs to be highly balanced in order to keep adequate contraction of cardiac muscle during the following cycles [28]. Finally, we investigated the slow effects on force development in the isometric mode of contraction (constant length) but the contraction in vivo is a combination of both the isovolumic phase and systolic ejection (cycled shortening–re-lengthening). Limiting our measurements to the isometric condition, however, allowed us to reveal shortening-independent effects of sudden stretch to Ca^{2+} homeostasis in cardiac tissue.

In conclusion, intracellular mechanisms of slow adaptation of myocardial contractility after sudden stretch are disrupted in failing rat myocardium, as demonstrated by the poor interrelation between the decay phase of the Ca^{2+} transient (governed predominantly by SERCA2a and Ca–TnC kinetics; the latter is assessed by the Ca^{2+} transient "bump" and "difference curve" methods) and the peak contractility. Metabolic dysfunction in the failing heart may contribute at least partially to the negative slow force response to stretch, in contrast to the substantial positive response observed in normal rat myocardium. The potential significance of this result is that the slow force response in diseased myocardium may be augmented under conditions affecting the kinetics of Ca–TnC interaction and/or Ca^{2+} extrusion from the cytosol.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the local institutional ethics committee.

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