Mathematical Modeling of the Effect of the Sarcoplasmic Reticulum Calcium Pump Function on Load Dependent Myocardial Relaxation

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Abstract. Earlier, we developed a mathematical model of myocardial contraction-relaxation cycle regulation. A great number of mechanical experiments was simulated in the model, the phenomenon of load dependent relaxation (LDR) included. In the present work we used the same model to analyze experimental data revealing that high temperature leads to reduction of LDR. We simulated three main factors arising due to high temperature, which *a priori* may cause LDR reduction: increasing the cross-bridges cycling rate, decreasing the duration of the Ca transient ascending limb, and increasing Ca pumping rate. Indeed, these factors together result in LDR reduction; i.e., the model correctly simulates the effect of high temperature on LDR in general. At the same time, the sensitivity of LDR to the third factor is much higher than to the first and the second ones; i.e., increasing the rate of Ca pumping is sufficient to induce the observed effect in the framework of the model. This seems to contrast with the result of our previous study dealing with the simulation of LDR disappearance due to increasing Ca pumping rate as it happens during relatively severe cardiac hypertrophy. However, the model analysis shows that the specific mechanism underlying the change in Ca pumping rate in either case is extremely important for the effect on LDR. Particularly, the model predicts that LDR will reduce if this rate increases due to enhanced ATP hydrolysis rate by the Ca pump; and *vice versa*, if this rate increases due to decreasing retroinhibition of the pump ATPase, it may result in LDR increase. Probably, but the first mechanism is operational due to high temperature and makes LDR to reduce, whereas slowing down Ca pumping due to increasing retroinhibition results in LDR disappearance during severe cardiac hypertrophy.

Key words: Muscle contraction and relaxation — Mathematical model — Calcium-troponin binding — Calcium transient — Sarcoplasmic reticulum

Introduction

This work aims to analyze the temperature effect on LDR by means of a math-

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ematical model. This analysis, in turn, helps to shed light on some mechanisms regulating the phenomena of load dependent relaxation as such. It is natural to suppose that two intracellular systems control this phenomenon. On the one hand, the phenomenon may be influenced by the system of cycling myosin cross-bridges; on the other hand, the time course of Ca^{2+} activation may also play a role. A much more complicated problem is to clarify the specific mechanisms of these influences.

For example, LDR was found to disappear during relatively severe myocardial hypertrophy (Lecarpentier et al. 1987). As presumed, this disappearance must be due to the slowing down of Ca^{2+} uptake by the sarcoplasmic reticulum (SR) in hypertrophied myocardium (Brutsaert and Sys 1989).

In general, one probable scheme explaining the LDR phenomenon may be presented as follows.

During isotonic twitch sarcomeres shorten to reach much smaller end-systolic lengths, and this occurs with higher velocities than during isometric twitch when the shortening is limited by the series elastic component stiffness.

It was shown with the help of the aequorin marker that the time course of the Ca transient was longer in isotonic than in isometric twitch (Lab et al. 1984). This data was interpreted as being the result of a decrease in troponin C affinity to Ca^{2+} with sarcomere shortening. In turn, this shortening deactivation inevitably results in a much faster relaxation phase. Thus, the LDR phenomenon might be controlled by the shortening deactivation of the thin filament.

Within the framework of this scheme, the smaller the afterload the more significant the LDR.

Moreover, it is clear that additional Ca^{2+} thrown off from the troponin C (TnC) during isotonic contraction as compared with the isometric one may either be bound with TnC once more or be pumped by SR. Therefore, the role of Ca pumping by SR may influence LDR.

The cross-bridges cycling effect on LDR is also implied in this scheme, because as we shall detail below, just this cycling determines reduction of TnC affinity to Ca^{2+} with sarcomere shortening.

Mathematical modeling is a useful method to study LDR. Moreover, we believe that it is absolutely necessary to use such modeling to study any effects connected with complex changes in different intracellular processes controlling mechanical properties of the cardiac muscle. Various influences changing LDR are just examples of these effects. It is clear that a mathematical model used to study effects affecting LDR in the heart muscle must, first of all, correctly simulate the LDR phenomenon as such and include descriptions of all the main intracellular mechanism underlying this phenomenon. For a long time we have been dealing with mathematical modeling of processes which regulate heart muscle contraction (Katsnelson et al. 1990; Izakov et al. 1991; Katsnelson and Markhasin 1996). In particular, special attention was paid to analysis of several aspects of the LDR phenomenon. A model of myocardial contraction we developed successfully simulates LDR and its disappearance due to slowing down Ca uptake in SR (Katsnelson and Markhasin 1996). Besides, the model was verified by simulating a great number of experimentally

observed mechanical phenomena (Izakov et al. 1991; Katsnelson and Markhasin 1996).

In creating the model several assumptions were made. All of them are based on experimentally observed muscle properties (Izakov et al. 1991). In particular, there are two cooperative mechanisms of Ca^{2+} binding with TnC:

– TnC affinity to Ca^{2+} increases due to the increase in the amount of cross-bridges strongly attached to the actin filament (type 1 cooperativity);

– TnC affinity to Ca^{2+} increases due to the increase in the concentration of Ca-TnC complexes (type 2 cooperativity).

The model includes mathematical formulas describing dependencies of the cross-bridge average probability to be strongly attached to the actin filament on the sarcomere length and on the velocity of its shortening/lengthening (Izakov et al. 1991). These dependencies were carefully verified (ibidem). Thus, the length and its change velocity influence the number of strongly attached cross-bridges in the model and hence – through the type 1 cooperativity – they influence also TnC affinity to Ca^{2+}. Just this feedback between mechanical conditions of the muscle twitch and its Ca^{2+} activation allowed us to simulate and explain LDR-phenomenon within the model (Katsnelson et al. 1990; Izakov et al. 1991). At the same time, these circumstances revealed that both Ca^{2+} activation and cross-bridges cycling through the cooperativity mechanisms actually controlled LDR within the model.

The next step of the model analysis was connected with the above-cited disappearance of LDR due to slowing down of Ca pumping during severe myocardial hypertrophy. As the analysis showed the slowing down should be very specific in this case: the Ca pump rate should be even smaller in the isotonic mode as compared with the isometric one; i.e., additional free intracellular Ca^{2+} thrown off from TnC due to the shortening in isotonic mode as compared with isometric one should result in additional slowing down of the pumping. Therefore, we have taken into account that, according to experimental data (Martonosi 1979), a partial inhibition of the SR calcium pump occurred alongside an increase in the amount of calcium uptake by SR. A modified formula of Ca pump describing this partial inhibition allowed us to solve, within the model, the problem of LDR disappearance due to slowing down of Ca pumping during severe myocardial hypertrophy. The following conclusion arrived: probably, the level of inhibition becomes higher in severely hypertrophied myocardium in comparison with normal one. This circumstance results in slowing down Ca pumping in both isometric and isotonic modes. However, in the latter case it is even stronger. Hence, LDR diminishes, right down to its absolute disappearance. These results and the underlying reasons were discussed elsewhere (Katsnelson and Markhasin 1996).

Thus, we have a positive previous experience in the application of the model to various aspects of LDR phenomenon. This made us employ the same model for an analysis of the temperature effect on LDR.

Different authors experimentally observed the dependence of LDR on temperature. For instance, data published by Pogessi et al. (1982) revealed this dependence. One of the most detailed experimental studies of this effect was carried out by Do-

brunz and Berman (1994). They showed that the increase in temperature from 24℃ to 37℃ leads to considerable attenuation of LDR in myocardial strips. Remarkably unlike in hypertrophied myocardium this attenuation was accompanied with an obvious increase in Ca pumping rate rather than its decrease. Dobrunz and Berman described (*ibidem*) a number of other mechanical events which also resulted from the above temperature change. In particular, they examined how this change influenced mechanical characteristics of the isometric contraction-relaxation cycle such as $T_{+\mathrm{d}F/\mathrm{d}t}$ (time to peak $+\mathrm{d}F/\mathrm{d}t$), $TR_{1/2}$ (time to half relaxation from peak force), and τ_{f} (time constant of the final exponential decay of force from 10% developed force). Using Q_{10} magnitudes Dobrunz and Berman showed that the change of temperature affected the decrease in τ_{f} to the greatest extent and the decrease in $T_{+\mathrm{d}F/\mathrm{d}t}$was affected to the least extent. The effect on $TR_{1/2}$ was intermediate.

Besides, the authors showed that the $T_{+\mathrm{d}F/\mathrm{d}t}/TR_{1/2}$ ratio increased with the increasing temperature. Dobrunz and Berman used these data to explain LDR diminution due to high temperatures. For this purpose, they considered $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$ and τ_{f} as indices of intracellular molecular mechanisms. Indeed, according to a variety of experimental data (Yue 1987) $T_{+\mathrm{d}F/\mathrm{d}t}$ coincides with the peak of the Ca transient, and these authors used this as an indirect indicator of the timing of SR Ca handling in general. Furthermore, they used τ_{f} as a pure indicator of cross-bridges kinetics (Peterson 1989) and $TR_{1/2}$ as a mixed indicator of both cross-bridges kinetics and Ca handling. Analyzing changes of these indices due to high temperatures they concluded that the decrease in $T_{+\mathrm{d}F/\mathrm{d}t}$ indicated acceleration of Ca uptake by the SR. However, they stressed that τ_{f} changed 3 times as large as $T_{+\mathrm{d}F/\mathrm{d}t}$. Therefore, they believed that just acceleration of cross-bridges detachment was the main determinant of the increased myocardium relaxation rate in the isometric mode. Moreover, they thought that the ratio $T_{+\mathrm{d}F/\mathrm{d}t}/TR_{1/2}$ excluded a contribution of Ca kinetics to the index $TR_{1/2}$ and therefore indicated only cross-bridges cycling. Thus, from their viewpoint an increase in this ratio also confirmed the key role of cross-bridges in accelerated isometric relaxation due to high temperature.

Their final conclusion is as follows. "Increasing temperature produces ... two competing and opposite influences on the degree of LDR: acceleration of the intracellular calcium transient which would by itself increase load dependence of relaxation, and acceleration of isometric relaxation which would by itself decrease load dependence of relaxation. The actual change in LDR with temperature reflects a balance of these two competing effects... Increasing temperature speeds up twitch mechanical characteristics to a greater degree than it speeds up SR calcium handling, resulting in the observed decrease in LDR". In other words, "Ca transient persists later into the isotonic twitch relative to the time course of isometric relaxation, consistent with the observed decrease in LDR".

Thus, the conclusion of the above authors was based on four main premises. First, they considered $T_{+\mathrm{d}F/\mathrm{d}t,}\, TR_{1/2}$ and τ_{f} to be indicators of intracellular processes as described above. Second, they believed that Ca pumping by itself mainly

controls isotonic rather than isometric relaxation. Third, they declared that acceleration of this pumping would always induce, by itself, an increase in LDR. Fourth, they believed that cross-bridges detachment mainly controls isometric rather than isotonic relaxation, and therefore acceleration of the detachment should induce by itself, just decrease in LDR. Furthermore, they compared these potential "increases" and "decreases" in LDR by means of the above indicator, and obtained a prevailing "decrease" in the case of increasing temperature.

However, we will try to show that their assumptions may be disputed.

In particular, the most important misleading point within the framework of the cited concept is the assumption that acceleration of the Ca transient always induces, by itself, an increase in LDR, because this acceleration mainly induces an increase in isotonic rather than isometric relaxation rate, whereas acceleration of the cross-bridges kinetics, on the contrary, mainly accelerates isometric relaxation and consequently induces, by itself, a decrease in LDR. As the model analysis shows, both these factors accelerate both isometric and isotonic relaxation. Such an alternative assumption seems more preferable, as it not only was verified within the model, but has also an independent experimental justification (Chemla et al. 1986) which we shall briefly clarify below. Evidently this alternative assumption completely rejects the cited concept.

Let's note that just the assumption that shortening of the Ca transient time course always induces an increase in LDR prompted Dobrunz and Berman to seek an opposite stronger mechanism which would lead to a final decrease in LDR due to high temperature despite the presumable effect of Ca pumping.

However, earlier published experimental data showed, on the contrary, that shortening of the time course of Ca transient due to a decrease in extracellular Ca^{2+} in solution resulted in LDR decrease (Chemla et al. 1986). Moreover, it did result in simultaneous acceleration of both isotonic and isometric relaxation with a prevailing increase in the rate of the latter one. These data mean that acceleration of the Ca transient not always produces, by itself, an increase in LDR.

On the other hand, the data on hypertrophied myocardium mentioned above suggest that acceleration and decreased Ca transient in normal as compared with hypertrophied tissue did result in elevated levels of LDR.

Probably, a decreased Ca activation may produce, by itself, both an increase and a decrease in LDR in various conditions, and the result depends on more specific features of this activation. From our point of view, the key feature is the difference between Ca activation of TnC in isotonic twitches vs such activation in isometric mode. If any factor acting on the muscle decreases this difference, LDR will also decrease, and *vice versa.*

As we shall show further in this work, a decrease in this difference may appear together with both generally increased and generally decreased Ca activation of the muscle. Moreover, the result may be controlled by various mechanisms responsible for the change of the Ca pumping rate. In particular, we will show here that acceleration of the pumping may induce, by itself, either increase or decrease in the above difference, despite the fact that any such acceleration certainly produces, by

itself, an accelerated Ca transient.

Therefore, acceleration of Ca pumping may, by itself, cause either increase or decrease in LDR. More exactly, at least in the framework of our model, acceleration of Ca pumping caused by acceleration of the pump ATP hydrolysis may lead to a decrease in LDR, whereas acceleration of the pumping due to weakening of the pump inhibition may lead to an increase in LDR. This is the main concept of this study which is analyzed and discussed in detail below.

Methods

Description of the mathematical model

As a tool for this theoretical study we used our mathematical model which has been published previously (Izakov et al. 1991; Katsnelson and Markhasin 1996). By means of that model we succeeded in explaining and simulating the effects of mechano-chemical uncoupling, LDR included (Izakov et al. 1991). Later on, we perfected the model so as to enable to simulate other physiological phenomena. In particular, we simulated and explained LDR disappearance which often accompanies the slowing down of Ca uptake by the longitudinal SR (LSR) in hypertrophied myocardium (Katsnelson and Markhasin 1996).

The model equations have been already substantiated and described in detail in the above mentioned papers. Therefore, we shall now highlight only those peculiarities of the model which make it useful as an instrument for studying various aspects of the LDR phenomena.

(I) The model has been set up as a system of ordinary differential equations in which all the variables represent either mechanical or biochemical characteristics of the muscle fiber.

(II) Such mechanical characteristics include: fiber length, internal length of the Contractile Element (CE), velocity of fiber shortening or elongation (and of CE shortening and elongation, as well), active and passive muscle tension.

(III) The concentration of attached cross-bridges is one of the variables responsible for the generation of active tension, and cross-bridges cycling is described in a particular differential equation of the model.

(IV) The concentration of calcium – troponin complexes (CaTn) is another variable of the model responsible for the generation of active tension. In the respective equation the model describes the process of calcium association with troponin (Tn) and dissociation of these complexes. The processes of calcium association – dissociation with other intracellular buffers, and Ca uptake by LSR are also described in the model.

(V) Two types of cooperativity of contractile proteins observed in biochemical experiments (Grabarek and Gergely 1983) take a central place in the model. They are included in the equation describing CaTn kinetics. The essence of this cooperativity is as follows:

i) the higher the concentration of myosin cross-bridges attached to the actin

filament close to the CaTn complex, the slower the dissociation of the complex (type 1 cooperativity);

ii) the higher the concentration of other similar CaTn complexes adjacent to that under consideration along the actin filament, the slower the dissociation of the complex (type 2 cooperativity).

(VI) Also, we described in the model a mechanism which, when combined with type 1 cooperativity, allowed us to find a feedback from the instant muscle length to the kinetic constant of CaTn complexes dissociation. That feedback is presented in the model by the formula $\Pi(n_1(l_1) \cdot n_2)$ (see Appendix 1, equation (2)), where Π is the function describing type 1 cooperativity; $n_1(l_1)$ is the average probability of a cross bridge "finding" an active site on a thin filament; l_1 is the instant length of the contractile element; n_2 is the average probability of the attachment of this cross-bridge to a site "found" (n_2 is described in a separate differential equation as its variable). All other details concerning this formula and its physiological grounds have been already given in our previous works (Izakov et al. 1991; Katsnelson and Markhasin 1996). The feedback from l_1 to the dissociation of CaTn leads, in turn, to additional inactivation of the fiber as a result of its shortening.

Items (V) and (VI) are the key points of our approach, which made it possible to explain and to simulate in the model both LDR and all the other effects of mechano-chemical uncoupling.

We present the model equations in Appendix 1 without any profound explanation of their physiological essence. Here, we would only like to stress that equation (3) from Appendix *1* describes cross-bridges kinetics, equation (2) the kinetics of CaTn, equation (4) the kinetics of intracellular free calcium. In the latter equation the rate of Ca uptake by LSR is given by the formula:

$$[r_{\mathrm{Ca}} \cdot \exp(-q_{\mathrm{Ca}} \cdot \mathrm{Ca_f})] \cdot \mathrm{Ca_f}, \tag{I}$$

where r_{Ca} and q_{Ca} are model parameters. Linear coefficient r_{Ca} characterizes the Ca pumping capacity, i.e. r_{Ca} reflects the rate of ATP hydrolysis in the Ca pump for active (uninhibited) calcium ATPase units. Exponential coefficient q_{Ca} characterizes the degree of pumping inhibition by calcium. We have already discussed in our previous paper all the reasons which had led us to involve this formula instead of the usual one describing the first order pump (Katsnelson and Markhasin 1996). The molecular mechanism underlying formula (I) is based on the finding that Ca^{2+} ion, being bound in LSR to the calcium ATPase (in the SR calcium pump), inhibits its activity (Inesi and Meis 1989). It was that particular formula that helped us to find an explanation for the LDR disappearance in hypertrophied myocardium observed experimentally in the guinea pig (Lecarpentier et al. 1987). As we showed earlier, this disappearance may be a result of specific slowing down of Ca uptake caused by increased inhibition of Ca pumping (Katsnelson and Markhasin 1996).

At last, we have to stress once more the role of the dependence of Ca activation on the muscle shortening. In our previous study we have already demonstrated in detail the great sensitivity of the presented model to changes in the sarcomere

length: sarcomere shortening produces earlier and faster relaxation due to type 1 cooperativity and to the effect of the sarcomere length on the above mentioned probability $n_1(l_1)$ (Katsnelson et al. 1990; Izakov et al. 1991). We have shown elsewhere the importance of this mechanism for the explanation of a number of phenomena simulated by the model (Izakov et al. 1991). As it will be shown in this work, the same mechanism turns out to be very important for the explanation of LDR decrease at higher temperatures.

Description of the numerical experiments

When simulating the changes in cardiac muscle behavior that result from its heating, we considered the same three changes considered by Dobrunz and Berman. Namely:

(α) growth of the rate of Ca pumping in LSR (in the case of heating, we realized this growth as an increase in the capacity r_{Ca}, *i.e.* as an increase in the ATP hydrolysis rate for uninhibited calcium ATPase units, rather than as a decrease in its inhibition. The reasons for choosing this particular variant of increasing the Ca pumping rate in order to simulate the phenomenon under study by means of the model will become clear from the analysis presented in Discussion below);

(β) reduction of the time of Ca transient's ascending limb (as a result of enhanced rate of Ca release from terminal cisterns);

(γ) increase in the cross-bridges cycling rate.

We could directly simulate all these changes by varying the values of the respective parameters of our mathematical model. Thus, we were able to find out whether the model reproduces LDR decrease when those three changes are simulated. Moreover, to understand which of these three factors is actually responsible for the phenomenon under study, we took the following steps in the numerical experiment.

Step 1. Simulation of a reference contraction (Figs. 1A and 2A) with the basic model parameter values as shown in Appendix 2.

Step 2. The capacity of Ca uptake by LSR was increased with respect to *Step 1* without any changes in other simulated muscle properties. To simulate this situation, we set the value of parameter r_{Ca} to 5-fold the baseline. All other parameter values were left unchanged, including parameter q_{Ca} (which expresses the inhibition of Ca pumping). Figs. 1B and 2B show the results of this step of the numerical experiment: the time course of muscle force development in isometric and isotonic regimes (Fig. 1B) and the corresponding muscle shortening (Fig. 2B).

Step 3. Figs. 1C and 2C illustrate the result of the stage of our numerical experiment where one (and only one) more parameter value was modified in addition to that performed at the previous step: shortening of the duration of the Ca transient's ascending limb (parameter t_d) from 75 ms to 30 ms.

Step 4. At this final step (see Figs. 1D and 2D), all factors (α), (β), (γ) were accounted for, because the rate of cross-bridges cycling was increased 10 times as compared to the previous step. For this purpose, we changed in a suitable manner

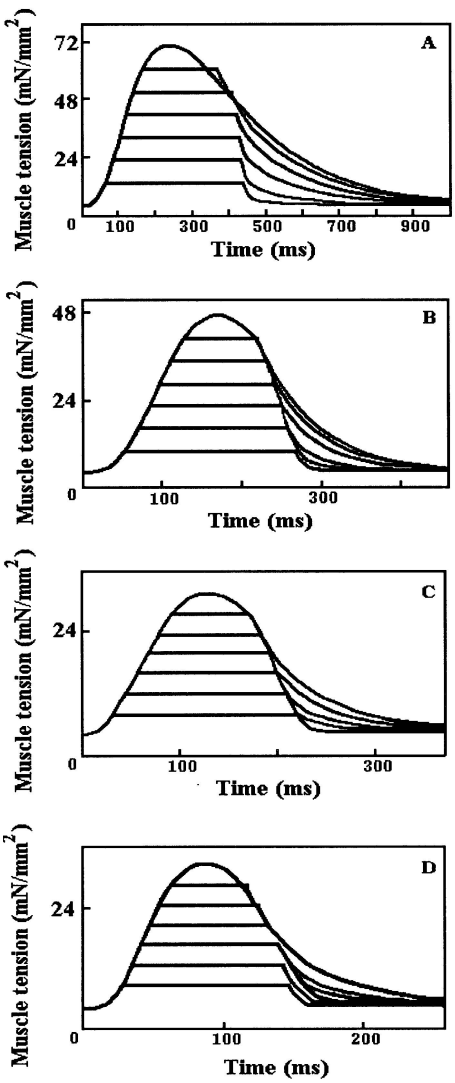

Figure 1. Simulation of the effect of cardiac muscle heating on LDR reduction. **Panels A÷D:** time course of muscle force generation in isometric and isotonic regimes. Following factors of the heating were consistently involved: (α) increase in the SR Ca pumping rate associated with an increase in ATP hydrolysis rate (parameter r_{Ca}); (β) reduction of the time of the Ca transient ascending limb (parameter t_{d}); (γ) increase in cross-bridges cycling rate (parameters of equation (3) from Appendix 1). **Panel A**: simulation of muscle contraction in its initial condition, *i.e.* before heating (basic parameter values from Appendix 2). **Panel B**: simulation of muscle contraction, (α) being the only factor involved. **Panel C**: factor (β) is involved additionally to the previous one. **Panel D**: all the factors (α), (β), (γ) are involved to simulate the effect of muscle heating on LDR.

the parameters of function $q_n(\dot{l}_1)$ from equation (3) (see Appendix 1). All the other parameters had the same values as at *Step 3.*

The selection of values for parameters t_d, r_{Ca}, and for those of function $q_n(\dot{l}_1)$ was driven by the values of Q_{10} established by Dobrunz and Berman for mechanical characteristics $TR_{1/2}$, τ_{f} and $T_{+\mathrm{d}F/\mathrm{d}t}$ reflecting changes in the molecular mechanisms under investigation caused by heating (increase in the Ca pumping, cross-bridge cycling and Ca transient rates). Specifically, in choosing values for the above parameters we sought to keep our simulations of contractions of a heated muscle

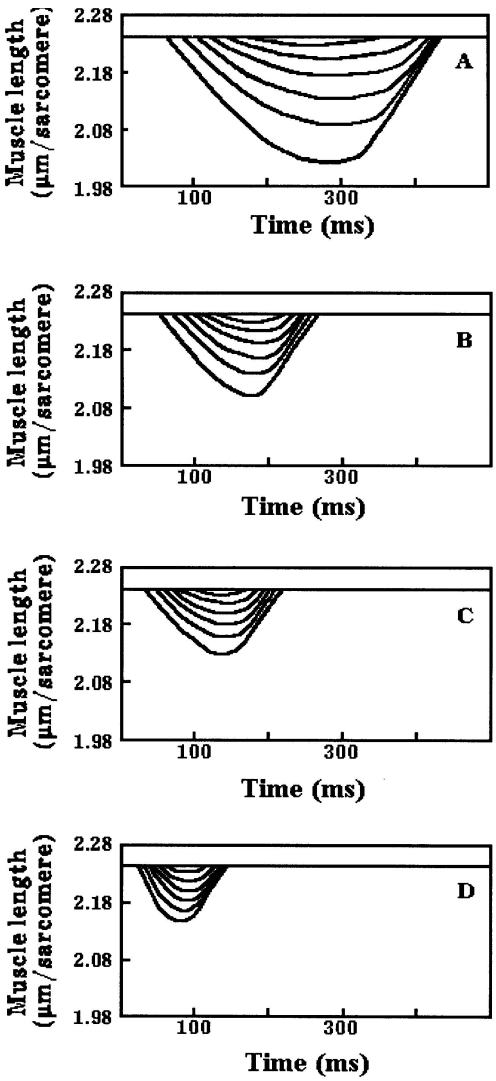

Figure 2. Simulation of the same muscle shortening and elongation in the same numerical experiments and in the same order as shown in panels of Fig. 1.

within the ranges of variations in characteristics $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$and τ_{f} reported by Dobrunz and Berman (Fig. 5 of Dobrunz and Berman 1994).

Thus, in the experiments carried out by Dobrunz and Berman, the value of $T_{+\mathrm{d}F/\mathrm{d}t}$ (time-to-peak of $\mathrm{d}F/\mathrm{d}t$) varied from 80 ms (± 20 ms) to 25 ms (± 5 ms) (see Fig. 5, Panel (a) of the paper cited) with an increase in temperature from 24℃ to 37℃. These ranges of $T_{+\mathrm{d}F/\mathrm{d}t}$ before and after heating determined our choice of the corresponding values for parameter t_d (time-to-peak of the Ca transient). The choice was correct because a close coincidence of t_d and $T_{+\mathrm{d}F/\mathrm{d}t}$

has been shown experimentally (Yue 1987). This choice does not change anything in our point of view explained in Introduction that both t_d and $T_{+\mathrm{d}F/\mathrm{d}t}$ are hardly representative indicators of the entire process of SR Ca handling. Particularly, it means that to simulate all the changes in Ca handling due to high temperature we have to separately determine the change of parameter r_{Ca} describing the rate of Ca pumping by SR.

We applied Q_{10} for $TR_{1/2}$ as a measure for assessing variation in r_{Ca} due to high temperature. However, it would not be correct to use $TR_{1/2}$ directly as this is a mixed indicator reflecting both cross-bridges cycling rate and SR Ca pumping rate. Therefore, we started from the independent assessment of the variation due to high temperature in the model parameters determining cross-bridges cycling rate, and used for this purpose τ_{f} as a direct indicator of this rate. Thereafter, we set new values of these parameters corresponding to the increased temperature; these conditions allowed us to find the necessary variation in r_{Ca} supplying required Q_{10} for $TR_{1/2}$ as compared with the initial state (Step 1) of the numerical experiment.

Our variation of the cross-bridges cycling rate was based on the following considerations. The model represents average probability n that the cross-bridge is in an attached position as $n = n_1 \cdot n_2$ (functions n_1 and n_2 are described in Methods and in Appendix 1). It follows that the rate constant representing the increase or the decrease in probability n during the twitch is given by:

$$\frac{\mathrm{d}n/\mathrm{d}t}{n} = \frac{\mathrm{d}n_1/\mathrm{d}t}{n_1} + \frac{\mathrm{d}n_2/\mathrm{d}t}{n_2};$$

i.e. this rate constant is given by the sum of the corresponding rate constants for probability n_1 and probability n_2. From the definition of these probabilities we see that the cross-bridge attachment/detachment process is directly connected with probability n_2 rather than n_1. The model should therefore represent the effect of temperature on the cycling rate as the variation in the rate constant $(\mathrm{d}n_2/\mathrm{d}t)/n_2$ for the purpose of increasing the latter. Namely, the parameters of function q_n from equation (3), Appendix 1 are responsible for the variation in this rate constant. In accordance with the above formula for the sum of rate constants, an increase in $(\mathrm{d}n_2/\mathrm{d}t)/n_2$ would bring about an increase, although a non-proportional one, in the rate constant $(\mathrm{d}n/\mathrm{d}t)/n$. This increase, in turn, would have an effect on the increase in the relaxation rate of the muscle. On the other hand, in accordance with the experiments carried out by Dobrunz and Berman, raising the temperature of the muscle from 24℃ to 37℃ should result in τ_{f} (time constant of the final exponential decay of force from 10% developed isometric peak) decreasing by about a factor of 8 (see Fig. 5, Panel (c) in the paper cited). Based on this consideration, we used this Q_{10} to estimate changes to be made in the parameters of function q_n (describing the rate constant for probability n_2) to simulate the behavior of the muscle at higher temperatures. The numerical experiment shows that an about eightfold decrease in τ_{f} is achievable by increasing the rate constant $(\mathrm{d}n_2/\mathrm{d}t)/n_2$ by a factor of 10 (See Results). This constant was thus increased in the model using such a tenfold increase in function q_n .

Finally, we calculated the sought-for parameter r_{Ca} change due to high temperature. In Panel (b), Fig. 5 of the above cited paper by Dobrunz and Berman, $TR_{1/2}$ (time to half relaxation from peak isometric force) falls from 255 ms ($\pm$35 ms) to approximately 35 ms with an increase in temperature from 24℃ to 37℃. We therefore chose to vary parameter r_{Ca} so that an increase in the Ca pumping rate ensured the required variation in quantity $TR_{1/2}$ as compared with the initial state (see Results). This turned out to be achievable by a fivefold increase in the baseline value of r_{Ca}.

We estimated LDR for each one of six afterloads $a_1, a_2, \ldots, a_6$ simulated in the above numerical experiments. As a measure of LDR, we used the same characteristic c/d applied in the experimental work mentioned (Dobrunz and Berman 1994), where c is the duration of the isotonic phase of the twitch under the given afterload a, and d is the duration of such part of isometric twitch where the force remains to be higher than a.

Results

When comparing Figs. 1A and 1B it is seen that factor (α) (*i.e.* the increase in the rate of Ca pumping by LSR) results in a considerable decrease in LDR. In fact, in Fig. 1A the values of the characteristic c/d for afterloads 1/7, 2/7, 3/7 are equal to: ~ 0.56, ~ 0.68, ~ 0.78, respectively (all the afterloads are normalized by the maximum active isometric force); whereas in Fig. 1B they are equal to: ~ 0.73, ~ 0.84, ~ 0.89 for the same normalized afterloads.

Furthermore, when both factors (α) and (β) (*i.e.* the increase in Ca pumping and reduction of the Ca transient's ascending limb) are taken into account, we obtain the following values of the characteristic c/d for the corresponding afterloads: ~ 0.73, ~ 0.84, ~ 0.93 (Fig. 1C). This means that in this case, LDR decreases with respect to the previous one only for afterload 3/7, and this decrease does not seem to be significant.

Finally, all three factors (α), (β), (γ) reflecting the muscle heating were taken into account concurrently at the step of our numerical experiment illustrated in Fig. 1D. The only change with respect to the previous experiment was an increase in the cross-bridges cycling rate. In this case, c/d for the corresponding afterloads takes on the following values: ~ 0.79, ~ 0.84 and ~ 0.97. It is seen that LDR again decreases slightly the afterload 3/7, remaining unchanged for all the other afterloads.

Characteristics $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$ and τ_{f}, which were equal to 72 ms, 250 ms and 300 ms at step 1, are now equal to 28 ms, 40 ms and 30 ms, respectively, i.e. $T_{+\mathrm{d}F/\mathrm{d}t}$ is now smaller by a factor of ~ 3, $TR_{1/2,}$ by ~ 6.3, and τ_{f}, by ~ 7.7, these characteristics thus being within the ranges of the variation reported by Dobrunz and Berman in the above cited paper. Note that it was not our intention to quantify muscle contractions based on the data reported by Dobrunz and Berman. Nevertheless, the values of $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$ as such (not only those of their Q_{10}) are

within the range of values reported by these authors (Fig. 5, Dobrunz and Berman 1994).

In addition to the numerical experiment described above we carried out a number of similar experiments with different "samples of muscles" simulated. In other words, these experiments were based on other sets of parameter values characterizing the initial state of a muscle before its heating. In general, we tested 30 "muscle's samples". For every "sample" all necessary changes of the model parameter values simulating the effect of temperature on LDR were calculated in accordance with the estimation method described in the previous section. Thus, we considered different magnitudes of changes in the parameters responsible for (α), (β), (γ) for all these variants, but varying these changes we confined ourselves to those only which did not take Q_{10} of characteristics $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$ and τ_{f} beyond the ranges set in section Methods. Furthermore, these changes were made in the same manner as for the basic sample presented in Appendix 2; i.e., factors (α), (β) and (γ) were made in sequence.

In addition, for all these "muscle samples" we varied all possible sequences of the heating factors, for instance:

1. $\{(\alpha)\}$, then $\{(\alpha)$ and $(\beta)\}$, then $\{(\alpha)$ and (β) and $(\gamma)\}$;
2. $\{(\gamma)\}$, then $\{(\beta)$ and $(\gamma)\}$, then $\{(\alpha)$ and (β) and $(\gamma)\}$;

etc.

The ranges of changes in characteristic c/d due to each of the factors (α), (β), (γ) obtained for the tested "samples" are shown in Table. The change in this characteristic was estimated as the ratio of its values before and after involving the corresponding factor.

Table 1. Comparative effects of factors (α), (β), (γ) on the characteristic c/d tested by means of 30 simulated "muscle samples".

Normalized afterload	Range of c/d changes due to factor (α)	Range of c/d changes due to factor (β)	Range of c/d changes due to factor (γ)
1/7	1.25÷1.45	1.0÷1.05	0.88÷1.05
2/7	1.20÷1.30	1.0÷1.04	0.89÷1.03
3/7	1.12÷1.20	1.0÷1.04	0.93÷1.03

All the above numerical experiments without exception confirmed that (α) was the predominating factor of LDR decrease among those studied. In very few cases, the contribution of factor (β) to the phenomenon discussed was comparable with that of (α). At the same time, the increase in the cross-bridges cycling rate (factor (γ)) has never influenced significantly the LDR decrease. Moreover, the experiment with the basic parameters as given in Appendix 2 but with another sequence of

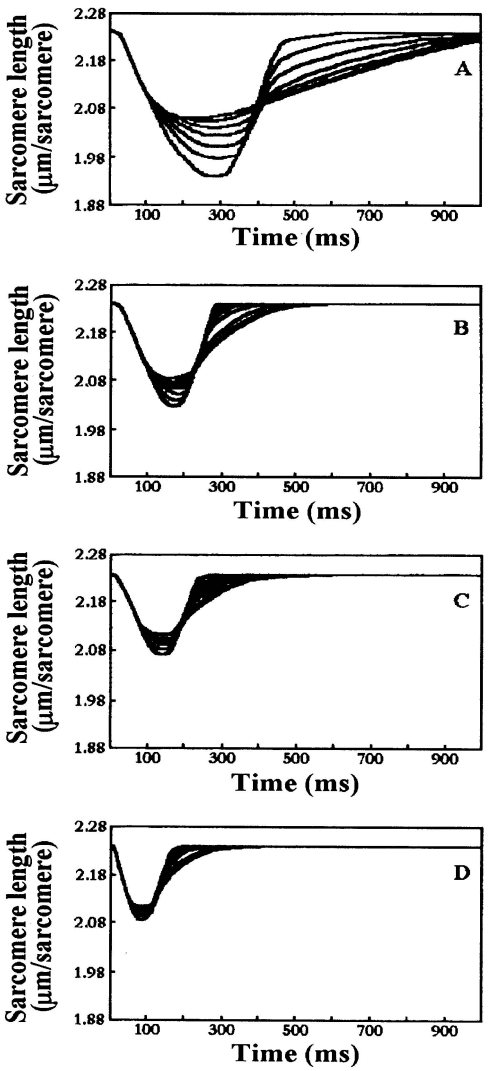

Figure 3. Simulation of the sarcomere shortening and elongation in the same numerical experiments and in the same order as shown in panels of Fig. 2.

involving the factors (namely, $\{(\gamma)\}$, then $\{(\beta)$ and $(\gamma)\}$, then $\{(\alpha)$ and (β) and $(\gamma)\}$) showed (see Fig. 4) that when (γ) was involved at step 1, LDR even increased slightly (c/d for afterloads 1/7, 2/7, 3/7 became equal to: ~ 0.50, ~ 0.61, ~ 0.74, respectively), and a considerable decrease appeared only at step 3, where (α) was involved as well.

Among others, we took (as "muscle samples") such basic sets of parameter values which provided for a lower level of cooperativity than the basic set presented in Appendix 2 (the latter corresponds to Panels A in Figs. 1, 2, 3, 4). As shown and

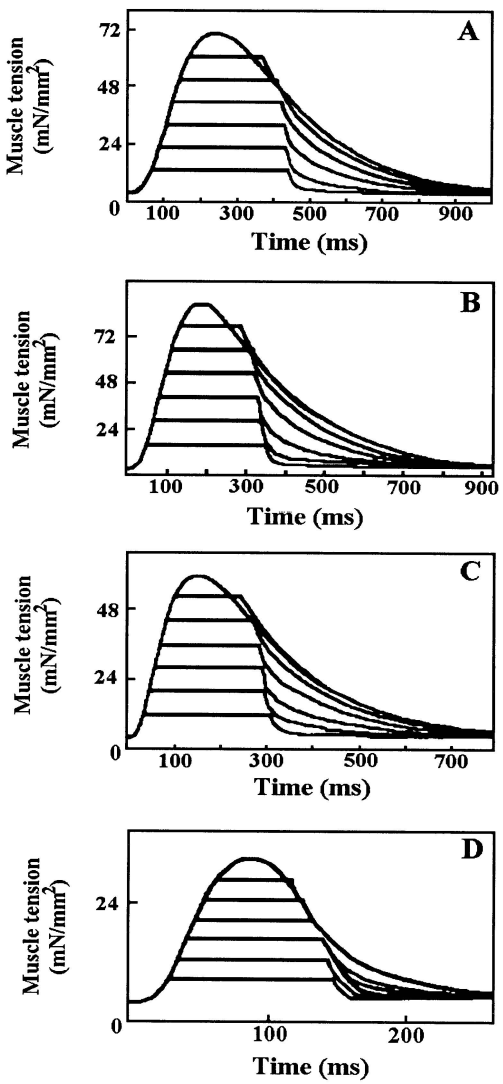

Figure 4. Simulation of the effect of cardiac muscle heating on LDR reduction (with the same baseline state but with sequence of involving the factors different from that illustrated in Fig. 1). **Panels A÷D**: time course of muscle force generation in isometric and isotonic regimes. Following factors of the heating were consistently involved: (γ) increase in cross-bridges cycling rate (parameters of equation (3) from Appendix 1); (β) reduction of the time of the Ca transient ascending limb (parameter t_{d}); (α) increase in the linear component of the rate of Ca uptake in LSR (parameter r_{Ca}). **Panel A**: simulation of muscle contractions in initial condition, *i.e.* before heating (basic parameter values from Appendix 2). **Panel B**: simulation of muscle contraction, (γ) being the only factor involved. **Panel C**: factor (β) is involved additionally to the previous one. **Panel D**: all the factors (α), (β), (γ) are involved to simulate the effect of muscle heating on LDR.

analyzed in one of our earlier papers (Izakov et al. 1991), a reduction in the level of cooperativity does, in itself, lead to a reduction in LDR. Herein we just repeat that experiment, introducing additionally factors (α), (β), (γ) which simulate heating against the background of a decreased cooperativity. The introduction of (α), (β), (γ) resulted in a considerable reduction in LDR, even against initially decreased cooperativity (hence, against low initial LDR), (α) remaining the dominant factor in this case as well.

The simulation of the total effect of temperature variations for all the 30

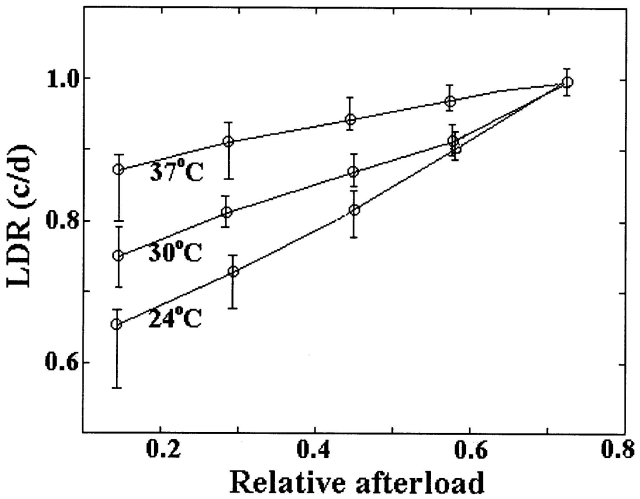

Figure 5. Simulation of the effect of temperature on LDR derived from a series of numerical experiments ($n = 30$). Values are mean ± sample standard deviation.

"muscle's samples" is shown in Fig. 5 where data obtained from this series of numerical experiments are summarized. In addition to LDR characteristics simulated for 24℃ and 37℃, we also included here a simulation of those for the intermediate temperature of 30℃. Qualitatively, the data very well correspond to those obtained by Dobrunz and Berman in a series ($n = 10$) of physiological experiments (Dobrunz and Berman 1994, Fig. 4). To simulate temperature transition from 24℃ to 30℃, we calculated the necessary changes in the values of model parameters in accordance with the estimation method described in the previous section; i.e., in this case we again estimated the changes by means of the values of $T_{+\mathrm{d}F/\mathrm{d}t}$, $TR_{1/2}$ and τ_f for 30℃, which are also presented in Fig. 5 of the cited paper by Dobrunz and Berman (1994).

Besides, we show here the influence of temperature on the effect of the magnitude of isotonic force on the rate of relaxation. Specifically, Fig. 3A shows how the length of the sarcomere changes, under initial conditions, in response to different afterloads, while Fig. 3D shows similar data for the last step of the numerical experiment allowing for all the three factors (α), (β), (γ), i.e. simulating the response of the muscle to an increase in temperature. In Fig. 3A the maximum rate of sarcomere elongation in "the muscle" undergoing relaxation reaches 2 μm/s for minimum afterload, and 0.26 μm/s for maximum afterload. The maximum rate of sarcomere elongation in such a "muscle" (Fig. 3D) is 2.23 μm/s for minimum afterload and to 1.17 μm/s for maximum afterload. Thus, in this numerical experiment simulating the mechanical behavior of the muscle in response to an increase in temperature, the above rates after the increase differ less than before the increase.

Discussion

A number of different mechanisms control muscle mechanical behavior, including LDR. The mathematical description of all the intracellular links underlying the mechanisms has been included in the model equations and discussed earlier (Izakov et al. 1991; Katsnelson and Markhasin 1996). Moreover, in the same works we studied, with the help of the model, the contribution of these mechanisms to the LDR phenomenon.

In this paper we deal with a relatively more specific problem using the same model: we try to clarify the main causes of LDR reduction due to high temperatures. For this purpose we, following Dobrunz and Berman, considered within the model the principal changes induced by increasing temperature in the intracellular processes responsible for the muscle mechanical behavior. We analyzed the influence of these changes on LDR. Namely, the following changes were considered:

(α) growth of the rate of Ca pumping in LDR due to the increased rate of ATP hydrolysis;

(β) enhanced rate of Ca release from the terminal cisterns resulting in an acceleration of the Ca transient ascending limb;

(γ) increase in the cross-bridges cycling rate.

Undoubtedly, each of these factors (α) $\div$ (γ) simultaneously influences several intracellular mechanisms, and through this influence affects LDR in an intricate manner. For example, factor (γ) inevitably causes changes of the shortening magnitude of the overlap zone length, of the shortening velocity, of the average load on one cross-bridge, and of the number of cross-bridges. Besides, through the cooperativity mechanisms, this factor changes intracellular Ca kinetics and consequently influences the time course of free intracellular Ca concentration and Ca handling processes. Factor (γ) induces these changes both in real muscle and in the modeled one. Moreover, we have to stress that in the modeled muscle all these changes occur automatically, when this factor is involved. It goes without saying that specific influences of factors (α) and (β) on the above six effects also automatically occur in the modeled muscle after involving these factors in the numerical experiment. As the influences of each of the factors (α) $\div$ (γ) are so complex and diverse, it would be difficult to predict *a priori* their final resulting effect on LDR. Numerical experiments in the mathematical model seem to be the most adequate method for carrying out this task. Exactly because of this, we analyzed in our numerical experiments both the individual role of the factors (α) $\div$ (γ) in LDR and their joint effect.

As numerical experiments show three factors (α), (β), (γ) reflecting muscle heating lead altogether to LDR reduction in the model (compare Figs. 1A and 1D), and this is in good agreement with the data from physiological experiments (Dobrunz and Berman 1994). However, the individual role of each of this factor seems (at least within the framework of the model) to contradict what was proposed by Dobrunz and Berman. In particular, when (and if) an increase in the cross-bridges cycling rate (factor (γ)) caused a decrease in LDR this effect was quite

small. Moreover, Fig. 4B shows an experiment where LDR even increased slightly as a result of involving factor (γ). In the meantime, factor (α) (*i.e.* the increase in the Ca pumping rate) revealed itself as a major determinant of LDR decrease (and, perhaps, as the only relevant one). In fact, all the numerical experiments pointed to factor (α) as being the predominating one. In very few numerical experiments the role of factor (β) was comparable with that of (α). Anyway, both these factors have a bearing to the increase in SR activity, while on the contrary, the increase in the cross-bridges cycling rate (factor (γ)) has never influenced significantly the LDR decrease.

The data from physiological experiments conducted by Dobrunz and Berman (1994) allowed them to conclude that the cross-bridges cycling rate increased more significantly than the rate of Ca uptake after muscle heating. Therefore, it was reasonable to follow the same in simulating the effect of heating: *in our numerical experiments we also used a higher increase in the cross-bridges cycling rate than in the Ca uptake rate.* The model studies show that this higher increase in the cross-bridges cycling rate is not as important a determinant of the decrease in LDR as the relatively smaller but nevertheless considerable increase in the Ca pumping rate. This fact does not seem to be surprising because the heart muscle is an essentially non-linear system.

There is one much more valuable divergence between these data and the idea of Dobrunz and Berman we showed in Introduction. Indeed, these authors believed that Ca pumping acceleration, by itself, always induces just an increase in LDR. From such a point of view a prevailing influence of the pumping acceleration as compared with acceleration of cross-bridges cycling would directly contradict the observed effect of LDR reduction due to high temperature. However, we already have argued that this viewpoint on the role of Ca pumping acceleration is not correct in general (see Introduction). Our numerical experiment confirmed this criticism.

Thus, the model shows that an increase in the rate of Ca pumping may lead to LDR reduction. It is the point of a principal importance that this is such an increase which is realized here as an increase in the rate of the Ca pump's ATP hydrolysis. Let us compare this result with the result of our previous paper (Katsnelson and Markhasin 1996), where the same model successfully simulated LDR disappearance, due on the contrary, to slowed-down Ca pumping by the LSR. However, in that work we revealed that this effect only results from a slow-down which is caused by enhanced tendency of Ca uptake inhibition. In terms of our model it means that, for LDR to disappear, an increase in the value of parameter q_{Ca}, rather than a decrease in r_{Ca} was the cause of the Ca uptake slow-down. Reversing this property of the pump we have to conclude that its acceleration due to decrease in the value of parameter $q_{C\mathrm{a}}$ would lead, by itself, to enhanced LDR rather than to LDR reduction.

That is why we propose here the following concept: one, and only one type of the increase in the Ca pumping rate of the two possible ones leads to LDR reduction, and just this, an increase due to acceleration of ATP hydrolysis, presumably

takes place when the temperature is increased. The second type, increase due to weakened pump inhibition, would lead to an increase rather than a decrease in LDR. Therefore, the second kind barely would take place when the temperature is increased. Below, we are discussing this concept in detail.

Thus, the model analysis shows that changes in Ca pump properties affect LDR in an intricate manner. This influence is not monotonous (in the mathematical sense): both an increase and a decrease in the rate of pumping may result in LDR reduction due to a specific mechanism responsible, in either case, for this change in the rate. In our previous work we have already discussed the mechanism connected with the enhanced tendency of Ca uptake inhibition (Katsnelson and Markhasin 1996). Later on in this section, we shall repeat some aspects of this discussion; but now we start by analyzing our new results.

We shall clarify how the increase in parameter r_{Ca}, which characterizes the uptake capacity of the Ca pump for uninhibited calcium ATPase units, influences LDR reduction as this influence was shown to take place in our numerical experiments.

First of all, as the model analysis shows, the phenomenon of LDR as such is a result of the peculiarities of isotonic rather than isometric twitches. Moreover, the phase of isotonic contraction predetermines LDR to a large extent. In fact, the more significant shortening of muscle sarcomeres in isotonic contraction as compared to isometric one results (due to type 1 cooperativity) in an additional decay of CaTn. Afterwards, not only does this difference reveal itself as a different start level of CaTn concentration at the beginning of relaxation, but it also influences the rate of dissociation of these complexes during the following phase of isotonic relaxation. This is so because both the instantaneous sarcomere length (due to type 1 cooperativity) and the instantaneous CaTn concentration (due to type 2 cooperativity) influence this dissociation not only instantly but afterwards as well. Furthermore, the smaller the level of CaTn in isotonic relaxation the smaller the level of muscle activation and, consequently, the faster the relaxation phase. It is to the point to stress here that in any twitch at the beginning of relaxation the level of CaTn is far from zero. This conclusion results even from the estimation of the CaTn course proposed by Peterson et al. (1991). Moreover, the estimate made by these authors seems to underestimate the real CaTn course because these authors tried to estimate the CaTn concentration at several moments of the twitch using the method of muscle short-time deformations. Such a procedure inevitably caused a considerable additional decay of CaTn. Thus, it seems probable that the muscle has to sustain a sufficiently high level of CaTn in the process of its relaxation essentially longer than the estimate by Peterson et al. We think the level predicted by our model might be more realistic (Katsnelson and Markhasin 1996).

So, we conclude that the difference in the shortening of the contractile element at the end of the contraction phase, between isotonic (under various afterloads) and isometric regimes, dramatically influences the time course of CaTn during the relaxation phases in these regimes, and results in LDR.

Evidently, we can conclude that *any factor contributing to the diminution of*

that difference is thereby directed at decreasing LDR. Factor (α) is just one of these.

Let us compare Figs. 2A and 2B which show the time course of muscle shortening in the initial step of our numerical experiment (Fig. 2A) and after r_{Ca} increase (Fig. 2B). In the first case, the shortening is much larger than in the second one. The reason for this difference is clear: increased Ca uptake by the LSR (whereas all the other parameters are left unchanged, that of inhibition (q_{Ca}) included) leads to the attenuation of the muscle's capability to shorten in the second case. It explains the LDR reduction observed in the model as a result of the increase in Ca pump capacity (for uninhibited calcium ATPase units).

The contribution of each of the factors (α), (β), (γ) to the above difference in contractile element shortenings between isotonic (under various afterloads) and isometric regimes is even more evident if we look at Fig. 3 illustrating the internal shortening of the sarcomere for the same contractions as shown in Figs. 1 and 2. As in the previous Figures, the sequence of Panels 3B, 3C, 3D corresponds to that of including factors (α), (β), (γ) in this experiment. In Fig. 3A, corresponding to the initial step of the experiment, the difference in sarcomere shortening between the isometric and isotonic regimes under minimal load is 0.12 μm (2.06 μm − 1.94 μm). The introduction of factor (α) reduces this difference to 0.04 μm (2.08 μm − 2.04 μm). The introduction of factor (β) results in the same difference (2.12 μm − 2.08 μm). Finally, the introduction of factor (γ) reduces it to 0.02 μm (2.11 μm − 2.09 μm). Thus, the introduction of factor (α) results in the greatest difference.

It is clear that any reduction in the difference in sarcomere shortening between isometric contraction and isotonic twitches is a circumstance which always supports the decrease in the difference in Ca activation between the isometric and isotonic mode. However, we have to stress that in some cases the influence of this circumstance on the Ca activation may be either partially or even entirely overwhelmed by another counteracting effect on the same activation which occurs in parallel and may be due to the same factors. Indeed, exactly this would appear, if the Ca pumping acceleration were caused by a decrease in its inhibition constant (parameter q_{Ca}) rather than by an increase in ATP hydrolysis rate (parameter r_{Ca}). In that case, the pointed difference in sarcomere shortening between isometric and isotonic modes would also decrease due to accelerated Ca pumping, but on the other hand, the same decrease in the pumping inhibition would entirely compensate for the influence of this circumstance. Below in this section, we will analyze this important mechanism in detail, but now we start our further discussion from the analysis of the case of increased r_{Ca} , involved as factor (α) in the above described numerical experiments. As these experiments showed there was no compensating effect in this case. Therefore, a decrease in the above difference in sarcomere shortening due to factor (α) does lead to the final decrease in difference in Ca activation between isometric and isotonic mode, and this leads to LDR reduction.

We have to point out the following detail. As the numerical experiment presented in Figs. 1, 2, 3 reveals, sarcomere shortenings in isometric and isotonic contractions are very close. Indeed, the difference between end-systolic lengths in these contractions is about 0.04 μm, i.e. $\sim 2\%$ of the initial sarcomere length. As

a result LDR disappears almost entirely in this numerical experiment. Exactly the same, almost complete LDR disappearance due to high temperature was observed in real physiological experiments (Dobrunz and Berman 1994).

However, Dobrunz and Berman did not measure internal sarcomere shortening in their experiments. Therefore, it may be asked whether or not this closeness of end-systolic sarcomere lengths in our numerical experiment actually reflects reality. If not, it would mean that our explanation of the temperature effect on LDR was but an artifact of the model. Probably, it is not so, and the above features of end-systolic sarcomere lengths are realistic. Let us pay attention to Fig. 6E to explain the cause of the close lengths obtained in the numerical experiment. This Figure shows how the elastic force of the series element in the model depends on the value of this element stretching. This particular dependence corresponds to the parameter values as shown in Appendix 2; i.e. the dependence was chosen to simulate just such a series elasticity which is specific for real physiological experiments in myocardial strips (see the reasoning in Appendix 2). This elasticity is mainly determined by the fastened ends of the muscle sample. It is typical that such a series elastic element is highly compliant within the range of relatively small applied forces (see Fig. 6E), but the element stiffness steeply increases with a further increase in force. Taking into account this peculiarity of the series elasticity, let us consider the effect of factor (α) on the sarcomere shortening during both isometric contraction and isotonic twitch. First of all, factor (α) decreases the level of intracellular Ca^{2+}; i.e. it leads to a decrease in muscle contractility (DMC). In isotonic twitches, this DMC manifests itself as an increase in the sarcomere end-systolic lengths as it is seen from Panel B of Fig. 3 compared with Panel A. In particular, under minimal afterload 1/7, the sarcomere shortened to 1.94 μm at the initial step of the experiment (Panel A) *vs.* 2.08 μm produced by DMC due to factor (α) involving (Panel B).

On the other hand, isometric shortening of the sarcomere is always entirely determined by two (and only two) causes: the isometric force amplitude and the internal compliance of the series elastic element. Therefore, to understand the effect of the same DMC on isometric sarcomere shortening in the numerical experiment, we have to consider how this DMC changes the amplitude of the isometric force and how the series element translates this change of the amplitude into the change of the end-systolic isometric sarcomere length.

Comparing panels A and B in Fig. 1, we see that DMC manifests itself in isometric contraction as the fall of the peak force from 68 mN/mm^2 (Panel A) to 42 mN/mm^2 (Panel B). In Fig. 6E, the upper dotted horizontal line corresponds to the force value of to 68 mN/mm^2 and the lower dotted horizontal line to that of 42 mN/mm^2. It is seen from this Figure, that the first force (68 mN/mm^2) applied to the series element produces its lengthening by 0.18 μm, and the second one (42 mN/mm^2) produces series element lengthening by 0.16 μm. In isometric conditions, the sum of the sarcomere length and the length of the series element always remains unchanged during the contraction-relaxation cycle. In our numerical experiment, this sum was 2.24 μm. Evidently, this fact exactly determined the isometric end-systolic sarcomere shortening in this experiment. Namely, the isometric peak force

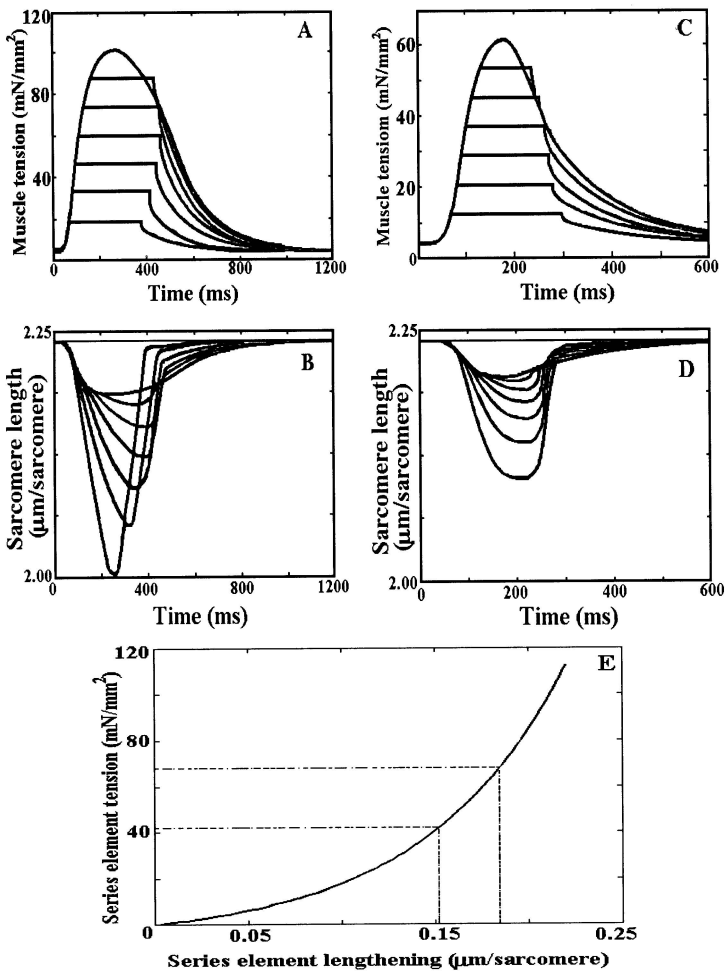

Figure 6. Simulation of the effect of the increase in r_{Ca} (factor (α)) on a muscle sample with a high series stiffness ($\alpha_1 = 21.0\ \mu\mathrm{m}^{-1}$, $\beta_1 = 40\ \mathrm{mN/mm}^2$). **Panels A** and **B** illustrate the muscle sample contractions in isometric and isotonic modes before involving factor (α). **Panels C** and **D** illustrate the same with factor (α) involved. **Panel E:** Dependence of the series element stiffness on its length for the basic parameter values of the model: $\alpha_1 = 13.3\ \mu\mathrm{m}^{-1}$, $\beta_1 = 6.4\ \mathrm{mN/mm}^2$.

of 68 mN/mm^2 produced a sarcomere shortening to 2.24 μm – 0.18 μm = 2.06 μm, whereas the isometric peak force of 42 mN/mm^2 produced a sarcomere shortening to 2.24 μm – 0.16 μm = 2.08 μm. Hence, in the case of a realistically modeled series elasticity for experiments in heart muscle strips, despite the great decrease

in isometric peak force due to increased rate of Ca pumping (parameter r_{Ca}), the corresponding change of the internal sarcomere shortening turned out to be very small. At the same time, as already mentioned, an increase in r_{Ca} manifested itself in isotonic contractions as a considerable decrease in the magnitude of sarcomere shortening. As a result, the end-systolic sarcomere lengths in isometric and isotonic contractions were so close after involving factor (α): 2.08 μm – 2.06 μm = 0.02 μm *vs.* 2.06 μm – 1.98 μm = 0.08 μm. Thus, our analysis shows that the non-linearity of the series elastic element in our numerical experiment was responsible for this strong effect of factor (α) with respect to muscle shortenings in isometric and isotonic conditions. The parameters of the series element, in turn, chosen for the numerical experiment were in accordance with the properties of the muscle strips' fastened ends typical of the real physiological experiments. Therefore, it should be expected that in such real experiments factor (α) (i.e. acceleration of ATP hydrolysis in the SR Ca pump) must yield the same close values of sarcomere shortenings during isometric and isotonic contractions.

One more conclusion may be drawn from the above reasoning: the properties of the series elastic element influence the effect of temperature on LDR. Therefore, the following questions arise. How does this effect depend on the degree of the series element's stiffness? How does this effect manifest itself in the intact heart, where compliance of the series elastic element is very small? In answering these questions, we have to note that internal sarcomere shortening during isometric contraction obviously must decrease due to the increase in the stiffness of the series element. And, even more, this must apply to both normal Ca pumping and pumping accelerated due to factor (α). Hence, differences in end-systolic lengths between isometric and isotonic contraction must be larger in this case both for initial conditions as compared to the baseline (Fig. 3A), and for conditions of factor (α) being involved as compared to the baseline (Fig. 3B). This means that, in the case of a more stiff series elastic element, LDR must be larger both for initial conditions as compared to the baseline (Fig. 1A), and after involving factor (α) as compared to the baseline (Fig. 1B).

Nevertheless, in the case of a more stiff series element factor (α) as such would also lead to some LDR reduction because of the same reasons as described for the usual, sufficiently compliant, series element. Thus, if the series element were more stiff, the involving of factor (α) would also result in LDR reduction; in this case, however, LDR would not almost disappear unlike in the case of the series element corresponding to the muscle strips' fastened ends. In Fig. 6, we show the results of a numerical experiment which thoroughly confirms the last statement. In this experiment, values of the stiffness coefficients α_1, β_1 were taken equal to 4.0 μm^{-1} and 210 mN/mm^2 instead of those presented in Appendix 2. The tension time-courses in isometric and isotonic modes before involving factor (α) are shown in Panel 6A, and those after involving factor (α) in Panel 6B. The values of the characteristic c/d for afterloads 1/7, 2/7, 3/7 change here from $\sim$ 0.47, 0.62, 0.75 to $\sim$ 0.58, 0.69, 0.84, respectively; i.e. LDR considerably drops due to factor (α) indeed, though it is far from LDR disappearance. Our conclusion that higher series

stiffness by itself increases LDR fits well with the observations of Donald et al. (1980). These authors were able to arrange the course of the muscle contraction so that the length of a controlled central muscle segment during the isometric phase of any contraction-relaxation cycle was constant. Such a method enabled them to eliminate the effect of the fastened ends compliance for the isometric phase of any contraction-relaxation cycle. This elimination corresponds to a much stiffer series elastic element than in the case of a fixed length of the whole muscle strip. These authors have shown that the elimination resulted in several changes, an increase in LDR included.

Let us note that the data of the numerical experiment represented in Fig. 6 make us suggest one more particular conclusion of a serious practical importance: the LDR phenomenon is to be operational for intact heart functioning in normal temperature conditions. This conclusion seems probable despite the cited experiments of Dobrunz and Berman who showed that LDR almost disappeared in rat, when the temperature became normal, physiological (37 ℃). In fact, the series stiffness in the intact heart myocardium is much higher than in experiments of Dobrunz and Berman dealing with myocardial strips *in vitro*. Therefore, it is likely that for the intact heart performance in the organism at 37 ℃ LDR should take place, as this is shown in Fig. 6B.

Now let us come back to the central point of our concept, i. e. to the statement concerning the predominating role of the intracellular calcium kinetics in different aspects of the LDR phenomenon, and particularly in the LDR reduction due to high temperature. This role is often masked because of a very complicated manner of its manifestations. First of all, it is related to the correlation between the change of the Ca transient and the change of the LDR level. For instance, Dobrunz and Berman (1994) state that Ca transient acceleration by itself always produces an increase in LDR. From our viewpoint it is not always so, because the experimental data we already have cited in Introduction (Chemla et al. 1986) revealed an example of the opposite situation. However, this example would hardly make us state that the shorter the time of the Ca transient and the lower the level of Ca activation, the less marked the LDR. Of course, such a statement would be incorrect as well. Our point of view on this subject is as follows.

Neither acceleration nor a slowing down of Ca activation due to some influence on the muscle is a real cause for LDR increase. The cause is a change that this influence produces in the difference between CaTn concentration in the isometric *vs.* isotonic mode. If this difference becomes smaller, LDR decreases. An increase in this difference in general may occur in parallel with either a total increase in Ca activation or a total decrease in it. And vice versa, if this difference becomes larger, LDR will increase despite the total decrease or total increase of Ca activation.

Let us illustrate this statement with the help of Figs. 7 and 8, where the data of the numerical experiment published earlier (Katsnelson and Markhasin 1996) are reproduced. These Figures show data of two numerical simulations by means of the same mathematical model used in the present paper. The data include time courses of the muscle force, shortening, CaTn concentration, and Ca transient.

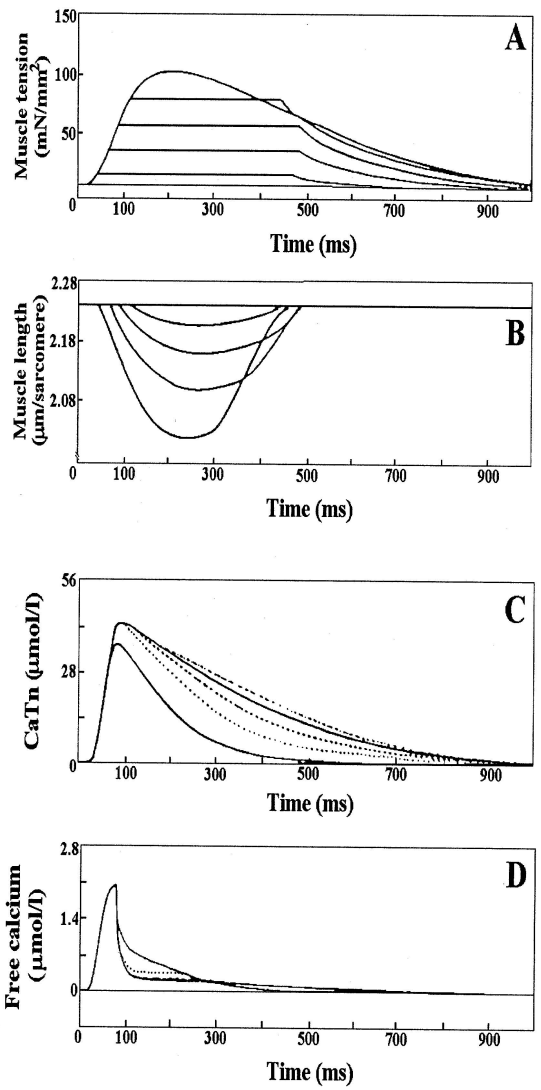

Figure 7. Modeling load-dependent relaxation of the heart muscle at $q_{Ca} = 50$. **Panel A**: muscle force generation in isometric (top trace) and in several isotonic (lower traces) contractions. **Panel B**: muscle shortening corresponding to these isotonic contractions: the less the after-load, the greater the shortening. **Panel C**: I – group of traces representing the course of CaTn concentration during the respective contractions. A lesser after-load results in visible inactivation of the process of CaTn association. II – group of traces representing the course of Ca binding with the buffer system in the same series of contractions. **Panel D**: Ca-transients corresponding to this series of contractions. The shortest Ca-transient corresponds to the isometric contraction, and in general, the less the after-load, the longer the Ca-transient. The data have been published elsewhere (Katsnelson and Markhasin 1996).

The only difference between the conditions of those two simulations was in values of parameter q_{Ca}: $q_{Ca} = 50$ for Fig. 7 *vs.* $q_{Ca} = 200$ for Fig. 8; i.e. inhibition of the SR Ca pump was stronger for the simulation shown in Fig. 8 as compared to that in Fig. 7.

When comparing panels 7C and 8C as well as 7D and 8D it is seen that the total level of Ca activation is higher for the case corresponding to $q_{Ca} = 200$. However, at the same time there are much more significantly expressed differences between isometric CaTn curve and isotonic CaTn curves in panel 7C as compared to the

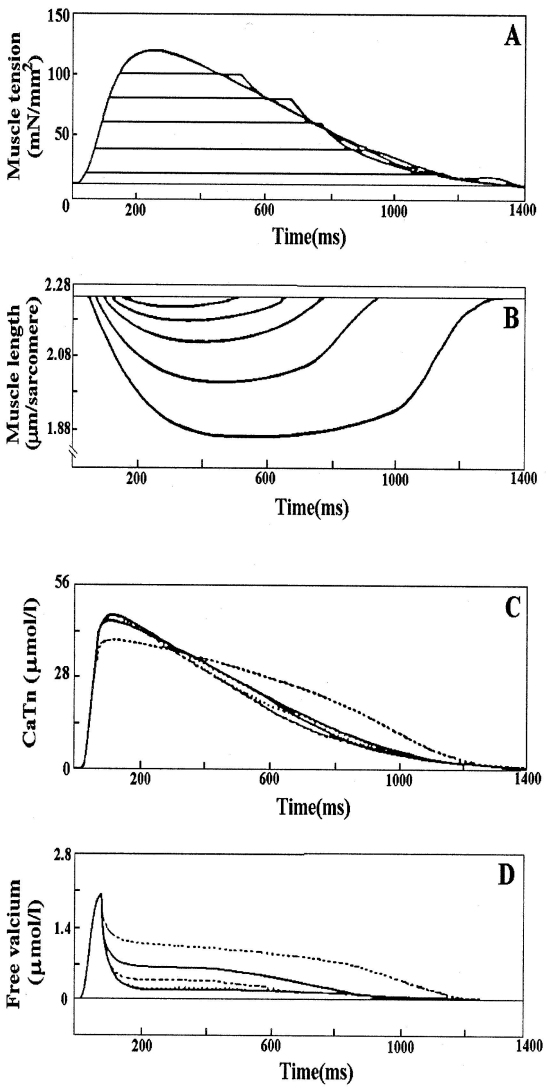

Figure 8. Modeling the disappearance of load-dependence for $q_{\mathrm{Ca}} = 50$. Unlike the processes shown in Fig. 7, here a lesser after-load results in a much more significant slowing down of the Ca-transient (**Panel D**), whereas inactivation of the CaTn association process is negligible. Moreover, after 200 ms (**Panel C**) an inverse pattern of the dependence of CaTn concentration on after-loads is seen. As a result, load-dependent relaxation disappears (**Panel A**). The data have been published elsewhere (Katsnelson and Markhasin 1996).

respective differences in panel 8C. LDR is seen in panel 7A but totally disappears in panel 8A.

To explain this result it is necessary to clarify how exactly the increase in SR Ca pump inhibition was manifested in the numerical experiments. On the one hand, the increase resulted in a total increase in muscle activation. This, in turn, caused more shortening in isotonic twitches, as can be seen in panel 8B *vs.* panel 7B. As already stressed, the constant of the CaTn dissociation permanently depends on the instantaneous muscle length through the cooperativity mechanisms. Hence, the

rate of CaTn dissociation in isotonic twitches became higher after increasing the inhibition constant ($q_{\mathrm{Ca}} = 200$) compared to isotonic twitches for the initial value of this constant ($q_{\mathrm{Ca}} = 50$). Let us take $\Delta_{\mathrm{F}}^{\mathrm{Ca}}(t)$ as the *additional* amount of Ca^{2+} which is dropped from CaTn at moment t in isotonic twitch under normalized afterload F as compared with the amount thrown from CaTn in the isometric mode at the same moment t. As it follows from the above, when $q_{\mathrm{Ca}} = 200$, then for any normalized afterload F and any moment t the instantaneous magnitude will be larger than $q_{\mathrm{Ca}} = 50$. However, this additional Ca^{2+}, for $q_{\mathrm{Ca}} = 200$ will be taken up by the SR Ca pump slowlier than at $q_{\mathrm{Ca}} = 50$. In other words, for $q_{\mathrm{Ca}} = 200$ this additional Ca^{2+} will be subsequently associated with TnC with a higher probability than for $q_{\mathrm{Ca}} = 50$. This circumstance explains why the differences between isometric and isotonic time courses of [CaTn] almost disappear in panel 8C. Moreover, for the minimum afterload shown in panel 8C the descending limb of [CaTn] proved to be even slower than for the isometric mode, whereas in panel 7C the corresponding descending limb for the minimum afterload just on the contrary forestalled isometric one. That is why an increase in inhibition of the SR Ca pump results in LDR disappearance, although the total level of Ca activation becomes higher due to this increase. Thus, the model analysis predicts that:

LDR depends non-monotonously (in the mathematical sense) on the rate of Ca pumping by LSR; i.e. LDR may diminish as a result of both an increase and a decrease in that rate, in accordance with the specific reasons causing these changes. In particular, LDR reduction results from both of the following changes:

– a decrease in the rate caused by enhanced Ca pumps inhibition (parameter q_{Ca});

– an increase in the rate due to the growth of ATP hydrolysis rate for the uninhibited calcium ATPase units in the SR pump (parameter r_{Ca}).

We think that the concept proposed in this work follows logically from our study. Indeed:

(I) on the one hand, neither an increase in cross-bridges cycling nor an increase in Ca pumping rate associated with a reduction in pumping inhibition resulted in the numerical experiments in a significant decrease in LDR (moreover, reduced inhibition as such resulted in a marked increase in LDR);

(II) on the other hand, another variant of Ca pumping rate increase, namely an increase in ATP hydrolysis rate, resulted in our numerical experiment in a decrease in LDR;

(III) in reality, LDR does significantly decrease with the increasing temperature as shown in physiological experiments by Dobrunz and Berman;

(IV) in reality, either the first or the second variant of the increase in Ca pumping rate should occur as well, because the rate actually increased due to increased temperature as shown in the same physiological experiments.

Comparing these four considerations it is clear to conclude (at least within the framework of the model) that if variant (II) of the increase in Ca pumping rate (increase in ATP hydrolysis rate) did not occur at the temperature increase, LDR reduction due to increased temperature would not occur at all. In other words, the

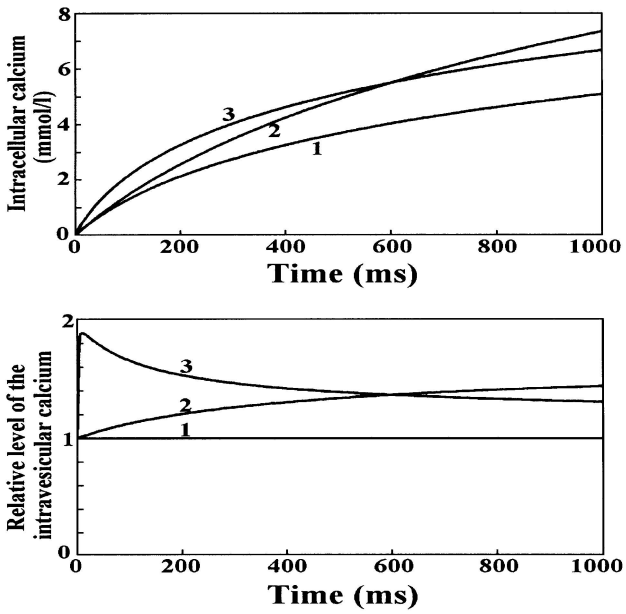

Figure 9. Simulation of the time courses of Ca concentration in the SR vesicles in different conditions. **Bottom panel.** Curve (1): $C_1(t)$, the time course of Ca concentration corresponding to the Ca pump basic parameter values. Curve (2): $C_2(t)$, the time course of Ca concentration corresponding to the following conditions: parameter r_{Ca} value (i.e. the rate of ATP hydrolysis in the pump) is 2 times the basic one; parameter q_{Ca} has the basic value. Curve (3): $C_3(t)$, the time course of Ca concentration corresponding to the following conditions: parameter q_{Ca} value (i.e. the level of the pump inhibition) is half the basic one; parameter r_{Ca} has the basic value. **Top panel** illustrates the same time courses 1, 2, 3 but in the normalized forms $C_1(t)/C_1(t)$, $C_2(t)/C_1(t)$, $C_3(t)/C_1(t)$, respectively.

latter variant of the Ca pumping rate increase seems to occur when temperature increases. This is but a hypothesis, of course, and we do not claim it to be more than this. Certainly, our suggestion that variant (II) occurs when temperature increases needs further experimental testing.

The question then arises whether it would be possible to design a biochemical experiment which can help to test this. To try and answer this question we simulated in the mathematical model the uptake of Ca by CR vesicles placed in a solution of constant concentration $[\mathrm{Ca_s}] = 2$ μmol/l. The initial concentration $[\mathrm{Ca_o}]$ in the vesicles was assumed to be 0.01 mmol/l. In this simulation, we used an equation for Ca pumping inhibited by intravesicular Ca. The upper panel in Fig. 9 shows:

– curve 1: $C_1(t)$ simulates the time course of the increase in calcium concentration in the vesicles for the basic values of the inhibition constant $q_{1\mathrm{Ca}}$ and ATP hydrolysis rate constant $r_{1\mathrm{Ca}}$;

– curve 2: $C_2(t)$ simulates the time course of the increase in Ca concentration in the vesicles for the same basic value of $r_{1\mathrm{Ca}}$ but for a reduced (compared with the basic value) inhibition constant $q_{2\mathrm{Ca}} = 0.5 \cdot q_{1\mathrm{Ca}}$.

– curve 3: $C_3(t)$ simulates the time course of the increase in Ca concentration in the vesicles for basic inhibition constant $q_{1\mathrm{Ca}}$, but an increased ATP hydrolysis rate ($r_{2\mathrm{Ca}} = 2r_{1\mathrm{Ca}}$).

$C_1(t)$ corresponds to the initial (basic) pumping characteristic; $C_2(t)$ corresponds to the pump taking up calcium faster than initially owing to reduced inhibition; $C_3(t)$ corresponds to the pump taking up calcium faster than initially owing to increased ATP hydrolysis. The lower panel in Fig. 9 shows curves for the ratios $C_2(t)/C_1(t)$ (curve 2) and $C_3(t)/C_1(t)$ (curve 3).

A comparison of $C_1(t)$, $C_2(t)$ and $C_3(t)$ shows that both methods of increasing the pumping rates ($C_2(t)$ and $C_3(t)$) lead to an increase in Ca concentration in the vesicles at any point of time as compared with the initial state $C_1(t)$, but it is rather difficult to find qualitative differences between curves $C_2(t)$ and $C_3(t)$. However, weighting $C_2(t)$ and $C_3(t)$ for the initial state $C_1(t)$ at each moment of time shows an important difference: the ratio $C_2(t)/C_1(t)$ is a monotonously increasing function, whereas the ratio $C_3(t)/C_1(t)$ has a marked peak at about the very beginning of the uptake process followed by a monotonous decay. This difference is not an accidental consequence of a fortunate choice of parameter values. We proved strictly a mathematical theorem stating that this qualitative difference between $C_2(t)/C_1(t)$ and $C_3(t)/C_1(t)$ is invariable with respect to changes in all the parameters: $[\mathrm{Ca_s}]$, $[\mathrm{Ca_o}]$, $q_{1\mathrm{Ca}}$, $q_{2\mathrm{Ca}}$ (provided $q_{2\mathrm{Ca}} < q_{1\mathrm{Ca}}$), $r_{1\mathrm{Ca}}$, $r_{2\mathrm{Ca}}$ (provided $r_{2\mathrm{Ca}} > r_{1\mathrm{Ca}}$). We do not think it appropriate to present the strict proof of the theorem here because this is not a mathematical journal. At the same time one important conclusion follows from this theorem: the difference between the ratios $C_2(t)/C_1(t)$ and $C_3(t)/C_1(t)$ can serve as a criterion applicable to any impact (including changes in temperature) on the muscle resulting in an increase in Ca uptake rate. This criterion enables to distinguish which of the two possible *a priori* variants (decrease in Ca pump inhibition or increase in ATP hydrolysis rate) underlies the above effect. Thus, in what follows we are suggesting a biochemical experiment with SR vesicles to provide an insight into which of the mechanisms increasing the Ca uptake rate comes into play when the temperature is increased. Two populations of vesicles are to be taken from a normal myocardium and the vesicles are to be loaded with calcium at two different temperatures T_1^{o} and T_2^{o} ($T_2^{\mathrm{o}} > T_1^{\mathrm{o}}$) while recording the concentrations of intravesicular calcium $\mathrm{Ca}_1(t)$ (at temperature T_1^{o}) and $\mathrm{Ca}_2(t)$ (at temperature T_2^{o}). The ratio $Z(t) = \mathrm{Ca}_1(t)/\mathrm{Ca}_2(t)$ should provide an answer to the question raised, namely if $Z(t)$ grows monotonously (within the range of non-saturating concentrations of calcium in the vesicles), this will provide evidence in favor of the hypothesis that inhibition reduces with heating. On the contrary, if $Z(t)$ has a marked peak at the initial stage of the Ca uptake process in the vesicles followed by a decrease, this will provide evidence that an increase in the Ca uptake rate in response to an increase in temperature is caused by growth in the pump's ATP hydrolysis rate. In other

words, this experiment would enable to confirm or to deny the main hypothesis of our work.

Appendix 1

Complete set of differential equations of the model

In their final (suitable for calculations) form the model equations are as follows:

$$\lambda \cdot p(\dot{l}_1) \cdot A_1^{\mu} \cdot n_2 \cdot n_1(l_1) \cdot (l_1 + S_0) = \beta_1 \cdot [\exp(\alpha_1 \cdot (l_2 - l_1) - 1] \tag{1}$$

$$\dot{A}_1 = c_1 \cdot \mathrm{Ca(t)} \cdot (1 - A_1) - c_{20} \cdot \exp(-q_k \cdot A_1) \cdot \Pi(n_1(l_1) \cdot n_2) \cdot A_1 \tag{2}$$

$$\dot{n}_2 = q_n(\dot{l}_1) \cdot [m(0) \cdot G^*(\dot{l}_1) - n_2] \tag{3}$$

$$\dot{\mathrm{Ca}}_\mathrm{f} = \begin{cases} 4a_\mathrm{c} \cdot \mathrm{Ca}_\mathrm{m} \cdot t \cdot [1 - \exp(-a_\mathrm{c} \cdot t^2)], & \text{when } t < t_\mathrm{d} \\ -A_1 - B - r_\mathrm{Ca} \cdot \exp(-q_\mathrm{Ca} \cdot \mathrm{Ca}_\mathrm{f}) \cdot \mathrm{Ca}_\mathrm{f}, & \text{when } t \geq t_\mathrm{d} \end{cases} \tag{4}$$

$$\dot{B} = b_\mathrm{on} \cdot (B_s - B) \cdot \mathrm{Ca}_\mathrm{f} - b_\mathrm{off} \cdot B \tag{5}$$

In isometric conditions one equation is added to the above ones, namely: $\dot{l}_2 = 0$.

In isotonic conditions it is replaced by the condition of constancy of the total afterload D on the sum of serial and parallel elements exhibiting exponential stiffness:

$$\beta_1 \cdot [\exp(\alpha_1 \cdot (l_2 - l_1)] + \beta_2 \cdot [\exp(\alpha_2 \cdot l_2) - 1] = D \ (= \text{constant})$$

The latter equality can be differentiated in order to reduce this case to a set of differential equations as well.

The force of the contractile element depends on the other model's characteristics as follows:

$$P_\mathrm{CE} = \lambda \cdot p(\dot{l}_1) \cdot A_1^{\mu} \cdot n_2 \cdot n_1(l_1) \cdot (l_1 + S_0)$$

Thus, the model presents a set of six differential equations with respect to six variables:

l_1 – length of the muscle contractile element;

l_2 – muscle length;

A_1 – CaTn concentration;

n_2 – average probability of a cross-bridge attaching itself to a vacant site "found" on the actin filament;

Ca_f – intracellular free calcium concentration;

B – concentration of calcium buffered by intracellular buffer system.

Other symbols are used in the equations in two cases:

(1) for variables which are expressed via these indicated six ones with the help of explicit functional dependencies (for instance, $n_1(l_1)$ is the probability of a myosin cross-bridge "finding" a vacant site on the actin filament; $p(\dot{l}_1)$ is the average force of an attached cross-bridge; and $q_n(\dot{l}_1)$ is responsible for the average rate constant of a cross-bridge attachment – detachment);

(2) for constants which are model parameters (for instance, λ, c_1, c_{20}, b_{on}, b_{off}, α_1, β_1, α_2, β_2, r_{Ca} , q_{Ca}, etc.).

Appendix 2

What follows is a list of the basic model parameters. The main part of the parameter values has been taken from our previous works (Izakov et al. 1991; Katsnelson and Markhasin 1996).

$\alpha_1 = 13.3\ \mu m^{-1}$
$\beta_1 = 6.4\ mN/mm^2$
$\alpha_2 = 14.6\ \mu m^{-1}$
$\beta_2 = 0.0048\ mN/mm^2$
$\lambda = 350\ (mN/mm^2)/\mu m$
$\mu = 1.7$
$S_0 = 1.14\ \mu m$
$b_{on} = 2\ ms^{-1}$
$b_{off} = 0.14\ ms^{-1}$
$B_s = 0.4$
$A_c = 0.001\ ms^{-2}$
$Ca_m = 0.03$
$T_d = 70\ ms$
$c_1 = 2.73\ ms^{-1}$
$c_{20} = 0.6\ ms^{-1}$
$\Pi_{min} = 0.02$
$q_k = 3.2$
$r_{Ca} = 0.7\ ms^{-1}$
$q_{Ca} = 40$

Parameters α_1 and β_1 are the exponential and the linear coefficient of the series elastic element, respectively. The characteristics of series elasticity were obtained in a fast muscle release experiment (Parmley and Sonnenblick 1967).

Parameters α_2 and β_2 are the exponential and the linear coefficient of the parallel elastic element, i.e. characteristics of passive stiffness of the myocardium. We estimated the values of these coefficients based on our own numerous experiments in rat papillary muscles (Markhasin et al. 1997).

λ is the scaler translating the force of one sarcomere into the force of the filament. We selected values for λ so as to provide (for predetermined values of series elasticity α_2 and β_2) a ratio between the amplitude of isometric tension and passive force that would fall within the ranges observed for L_{max}. The basic value of λ meets this condition (See, for instance, Fig. 1, Panel A).

S_0 is an absolute term in the linear dependence of overlap zone on sarcomere length S_0 calculated on the basis of the classical data on the characteristic sizes of thick and thin filaments and overlap zone obtained by optical methods (Gordon et al. 1966).

B_s, b_{on}, b_{off} are kinetic characteristics of the generalized intracellular Ca buffer. The values of these characteristics were obtained in a separate work of ours based on data on calcium binding ligands found by other authors in experiments in single cardiomyocites (Sipido and Wier 1991). This mathematical analysis and its results (numerical values of the above characteristics for the generalized buffer) were published by us elsewhere (Solovyova et al. 1997).

Parameters a_c, Ca_m, t_d refer to the upward phase of the Ca transient. We set this phase with the help of an explicit function borrowed from the model suggested

by Panerai (1980) and modified insignificantly. In this case, the value of coefficient a_c was chosen so as to ensure a steep increase in intracellular Ca concentration up to t_d, the time to peak of the Ca transient. The value of $t_d = 70$ ms was chosen because it falls within the range of values for the Ca transient peak time at 24°C as estimated by Dobrunz and Berman (1994) by measuring the value of $T_{+dF/dt}$ in mechanical twitches.

Ca_m is the amplitude of the Ca transient, for convenience expressed in the model as a dimensionless quantity in fractions of concentration TnC in the cell (dimensionless in the model are also all the instantaneous values of the concentrations Ca, CaTn and Ca-buffer ligands). The dimensionless value $Ca_m = 0.03$ given in the list of the basic values if expressed in micromols is equal to 2 μmol/l, which is somewhat higher than that calculated by Sipido and Wier based on experiments using Fura-2 fluorescence (Sipido and Wier 1991). In the experiments conducted by these authors maximum intracellular Ca concentrations varied from ~ 1 μmol/l to ~ 1.5 μmol/l. However, we took into account that the Fura-2 fluorescence marker used in those experiments is an additional calcium-binding buffer not available in the cell in natural conditions, which means that the natural concentration of free intracellular Ca will be somewhat higher than that observed in experiments with Fura-2.

c_1 is the CaTn complex binding rate, while c_{20} is a coefficient appearing in their decay constant (see Appendix 1, Equation (2)). Constant c_1 is made dimensionless in the same manner as quantity Ca_m. In dimensional concentrations it is equal to 39×10^6 $M^{-1} \cdot s^{-1}$, which is in agreement with data reported by Fabiato (1983). The value of c_{20} was chosen so that the range of changes in the decay constant in twitches (taking into account the cooperativity mechanisms) included the interval $230 \div 350$ s^{-1}. We borrowed this reference interval from the data of biochemical experiments in solution (Wang and Leavis 1988).

Parameters μ, Π_{min}, q_k refer to the formula describing the cooperativity mechanisms. Grounds for the introduction of these mechanisms into the model and the choice of the relevant parameter values were provided in our earlier studies (Katsnelson et al. 1990; Izakov et al. 1991; Katsnelson and Markhasin 1996).

Parameters r_{Ca}, q_{Ca} appear in the formula describing the Ca pump of the SR, where r_{Ca} is a linear coefficient while q_{Ca} is an exponential one. The meaning of these parameters has been discussed in detail above. The choice of a corresponding formula to describe the Ca pump in the model was discussed in our preceding paper (Katsnelson and Markhasin 1996). The basic values of parameters r_{Ca}, q_{Ca} were chosen so as to bring Ca pumping rates in the model in good agreement with the experimental data reported by Haynes and Mandveno (1987).

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