Effects of Mechanical Interaction Between Two Rabbit Cardiac Muscles Connected in Parallel

V. S. Markhasin[1], L. V. Nikitina[1], S. M. Routkevich[1], L. B. Katsnelson[1], E. A. Schroder[2] and B. B. Keller[2]

1 *Russian Academy of Sciences, Institute of Microorganisms' Genetic and Ecology, Ekaterinburg, Russia*

2 *University of Kentucky, Department of Pediatrics, Division of Pediatric Cardiology, 800 Rose Street, Room MN472, Lexington, KY 40536-0298, USA*

Abstract. The hypothesis that myocardium mechanical inhomogeneity produces a substantial effect on mechanical function was tested. Muscle inhomogeneity was studied in isolated papillary muscles or trabeculae excised from rabbit right ventricle and connected in a parallel duplex. Each muscle was placed in a separate perfusion bath. One end of each muscle was fastened to an individual force transducer and the other to the common lever of a servomotor. This arrangement allowed both muscles, being excited independently, to pull jointly a load applied to the lever. Separate electrodes for each perfusion bath allowed to stimulate muscles with a time delay. Tension developed in the individual muscles and their interaction were studied. Developed tension was critically dependent on the timing and sequence of excitation. Using mathematical modeling, patterns of tension distribution experimentally observed in parallel duplexes were simulated. These results suggest that changes both in Ca^{2+} transients and in the time course of Ca^{2+}-troponin complexion due to the duplexed muscles interaction offset the effect of mechanical inhomogeneity.

Key words: Myocardium — Inhomogeneity — Asynchronism — Mathematical modeling

Introduction

Cardiac muscle is non-uniform. The scale of this non-uniformity ranges from molecular (Noble et al. 1983; Samuel et al. 1983; Whallen 1985) to macroscopic, covering whole segments of heart chambers (LeWinter et al. 1975; Hosino et al. 1983; Markhasin et al. 1994). Using various methods including echocardiography (Haedchen et al. 1983; Pandian et al. 1983), contrast ventriculography (Klausner et al.

Correspondence to: Larissa V. Nikitina, Ph.D., Russian Academy of Sciences, Institute of Microorganisms' Genetic and Ecology, Pervomayskaya str. 91, Ekaterinburg 620219, Russia. E-mail: lbk@efif.uran.ru

1982; Sheehan et al. 1983; Markhasin et al. 1994), and nuclear magnetic resonance (Azhari et al. 1992), researchers have demonstrated substantial variability in the magnitude of deformation of various segments of heart chamber wall during systole. Numerical experiments using mathematical models predict that differences in force exist in the various layers of the myocardium, ranging from endo- to epicardium (Huisman et al. 1980; Beyar and Sideman 1987).

Inhomogeneity of the myocardial wall increases appreciably during pathologic states. Ischemic and infarcted areas in the heart chambers change their kinetics during systole (Tennant and Wiggers 1935). The ability of the myocardium to shorten changes both in the affected areas and in those remote from the focus of damage (Gallagher et al. 1986). In addition, segmental inhomogeneity of the myocardium substantially influences left ventricular systolic (Markhasin et al. 1994) and diastolic function (Lew and Rasmussen 1989; Schafer et al. 1992).

The study of properties of mechanical inhomogeneity could widen the paradigm of cardiac biomechanics (Katz 1988; Katz and Katz 1989) and help to understand the contribution of mechanical inhomogeneity to the heart regulation both in norm and pathology.

It is extremely difficult to assess the fundamental impact of mechanical inhomogeneity on myocardial ino-, lusi- and ergotropic functions in the intact heart due to its complexity. To resolve this problem, it is necessary, at least in preliminary studies, to use simplified inhomogeneous muscle systems as models of the inhomogeneity phenomena in the intact heart. We presume that any real complicated inhomogeneous system of any level, from cellular one to the entire heart, is a composition of the simplest inhomogeneous sub-systems, each being represented by the duplex, i.e. by two elements connected either in parallel or in series. Such representative duplexes in various cases may be either (i) elementary pairs (two adjacent interacting fibers or even sarcomeres) or (ii) two parallel segments within the ventricle circular layer or (iii) specifically for pathological conditions, pairs consisting of entire normal and abnormal myocardial segments (e.g. an ischemic segment interacting with an adjacent normal one).

A duplex muscle system we use for a simplified *in vitro* model of non-uniform myocardium is composed as follows. Two papillary muscles from the right ventricle of a rabbit, or two trabeculae, were removed and connected in parallel. These two muscles might differ from each other by a number of characteristics, including time to peak isometric force, isometric force amplitude, maximum muscle length ($L_{\max}$), unloaded shortening velocity ($V_{\max}$), shortening magnitude under a given normalized afterload. All these differences occur in intact heart in these or other types of inhomogeneity. For example, parallel segments in the circular layer may differ in the length (due to the different curvature), in the time to peak isometric force, and in $V_{\max}$. Besides, adjacent infarcted area and normal region interacting in the heart may also have different length and shortening magnitudes, etc.

Initially, mechanical characteristics of each of the muscles contracting individually were recorded. Next, the same characteristics were recorded when the muscles contracted within the duplex. In this way we were able to estimate the mechanical

effects of the muscles on each other. Moreover, by varying the mechanical characteristics of the muscles in the duplex physically and pharmacologically the effect of mechanical non-uniformity on the duplex as a whole was estimated.

Mathematical modeling was applied to clarify the possible mechanisms underlying the effect of non-uniformity on mechanical function of the myocardium. Specifically, we simulated interactions between two muscles connected in a parallel duplex. Model analysis enabled us to trace the effect of mechanical non-uniformity on the kinetics of free intracellular Ca^{2+} and Ca^{2+}-troponin complexes in a non-uniform myocardial system. We had previously published some results of theoretical analysis of inhomogeneous muscle duplexes obtained by means of a mathematical model (Markhasin et al. 1997a). However here, as compared to the mentioned study, we applied a more profound version of the mathematical model (Katsnelson and Markhasin 1996) for describing elements of the inhomogeneous duplex. In particular, the role of calcium uptake by the sarcoplasmic reticulum in regulation of myocardium contraction was taken into account in this version.

This paper illustrates how the distribution of the time course of force in each of the elements of an isotonically contracting muscle duplex is modified by this interaction, and how it responds to delays in the excitation between two muscles. The duplex mathematical model demonstrates a possibility to simulate adequately both the mechanical behavior of duplex members and associated changes in the kinetics of free intracellular Ca^{2+} and Ca^{2+}-troponin complexes.

Materials and Methods

Muscle Preparation

Experiments were performed on rabbits whose weight ranged from 1.5 to 2 kg. Before experimentation rabbits received heparin (0.3 ml/kg i.v.) to prevent clot formation and pentobarbital Na (30 mg/kg i.v.) as an anesthetic. The chest was opened and the heart rapidly removed and washed with a buffer solution which contained (in mmol/l): NaCl, 137; KCl, 2.5; $MgCl_2$, 1.0; $CaCl_2$, 2.5; glucose, 4.0; Trizma HCl, 10; and Trizma base, 10. The pH of the solution was 7.3 ± 0.05 at a temperature in the range from 28 to 31 °C. The buffer was bubbled with O_2. Two papillary muscles or thin trabeculae were then excised from the right ventricle. Preparations were 3–5 mm long with a cross-sectional area between 0.5 and 0.7 mm^2.

Muscle preparations were placed in dual 5 ml perfusion baths each of which contained a pair of platinum electrodes for electric field stimulation. One end of each preparation was fixed to the lever of a custom servomotor common for both muscles, and the other to a custom force transducer, which operated independently for each muscle. Shortening of the muscles in the duplex was common, but each of the muscles developed its own force.

In all experiments, preparations were stimulated at a frequency of 0.3 Hz by rectangular pulses of 5 ms duration exceeding threshold by a factor of 1.3. All

preparations were initially stretched to the length at which maximal isometric force was obtained ($L_{\max}$) and stimulated for an hour until contractions reached steady-state amplitude. The stretching of every preparation was carried out by means of separate micro-screw. We stretched that end of the sample which was directed to the force transducer, i.e. that one which then remained motionless during all further movements of the sample. Before each experiment the length of the muscle was set at 0.95 $L_{\max}$. For all preparations the passive to active force ratio $[F_{\mathrm{pe}}/F_{\mathrm{se}}]$ was determined at 0.95 $L_{\max}$. The preparations were considered suitable if the $[F_{\mathrm{pe}}/F_{\mathrm{se}}]$ ratio did not exceed 0.15.

The amplitude and the time to peak force (TPF) of the isometric contractions were monitored throughout the experiment (4–5 hours). Results of experiments with amplitude variations exceeding 5 % and with TPF variations exceeding 3 % were not accepted for the final analysis. 36 muscle duplexes from 48 rabbits were successfully studied.

Experimental Processes

A block diagram of the experimental setup is shown in Fig. 1.

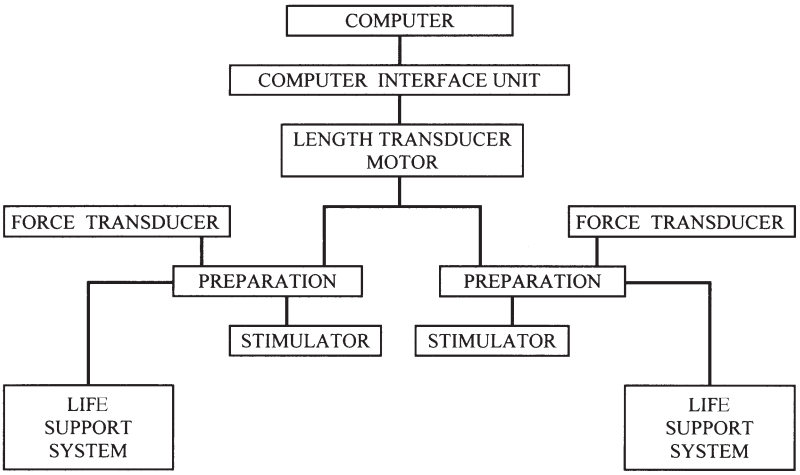

Figure 1. Block diagram of the setup.

A custom software package was developed for the protocols used during each experiment. These programs enabled an automated experiment to be performed on two preparations connected in parallel. The protocol included recordings of passive length-force relationships, an isometric protocol for different lengths of the preparations, an isotonic protocol, and a physiological protocol. The physiological protocol contained a physiological sequence of mechanical loads and allowed reproduction

of the mechanical loading phases which occur in the intact heart. Afterload was computed as a portion of the maximal force (F_0) with an increment of 0.1 F_0.

The parallel elastic element (PE), series element (SE), and contractile element (CE) are components of the classical three-element model of a cardiac muscle. All of our experiments were performed at the length 0.95 $L_{\max}$. The passive force developed by the muscle, which is associated with the PE, was only allowed to reach 15 % of the developed active force.

Passive length-force characteristics were determined by shortening the length of the duplex preparations to zero passive force and then returning to initial levels in the absence of stimulation. Since the PE has a viscoelastic nature, force of the PE depends not only on the length but also on the velocity and direction of length changes. The length-force relationship for the PE should be recorded during changes of length at approximately the same velocity and in the same direction as occurs *in vivo*. Though CE is not fully compliant even in an unstimulated muscle, its residual stiffness is so small that it may be neglected when specifying experimentally "passive deformation – passive tension" relationship for the PE. The force and length of both preparations were recorded during shortening. Filtering was applied to eliminate the effect of noise on the dependencies obtained. This line of testing was used to establish the working length of preparations, as well as for the elimination of the PE role.

In particular we were then able to apply our method (Markhasin et al. 1997b) of eliminating the PE role. The dependence $F_{\mathrm{PE}}(L)$ of the passive force on the length was previously recorded. This enabled us to set an algorithm of muscle loading for each current length L. Load on the muscle, $F(L) = d + F_{\mathrm{PE}}(L)$, where d is a constant. The CE is then under the same constant load d. The elimination of the PE role alters the slope of the length-force and force-velocity relationships. The method has been used both for every muscle of the duplex in individual contraction and for the duplex as a whole. Described methodology permits mechanical characteristics, for example length-force and force-velocity relationships to be estimated for the CE.

The setup calibration program ensured the calibration both of the force and length transducers and of the temperature-sensitive element. A custom data processing program was used to analyze the time course of force development and changes in the length and rate of shortening during isometric, isotonic and physiological regimes. The program produced graphic and tabulated representations of the inotropic and lusitropic characteristics (such as the length-force, force-velocity, performance-shortening, characteristic relaxation time-shortening) of the muscles contracting in isolation, within a duplex and as a duplex pair.

Mathematical Modeling of Muscle Duplexes

Main problems in duplex modeling are associated with the choice of a suitable basic uniform fiber model. Therefore, mathematical modeling of non-uniform muscle duplex contractions should be based on a carefully designed model of an uniform myocardium. The mathematical model of an uniform myocardium is in fact based

on an elementary contractile structure, i.e. a sarcomere placed in an appropriate rheological medium (Appendices 1, 2). It is sufficient to mathematically describe a parallel connection of two dissimilar sarcomeres (simulated by assuming various values for the model parameters) and to watch the effects of this connection in numerical experiments. In comparison with a physiological experiment, modeling allows us to observe how contractile protein activation processes vary in response to redistribution of loads, and to estimate the role of these changes on the function of the non-uniform duplex system. It is especially important that this model correctly reflects the feedback between the mechanical characteristics of a contracting uniform fiber and its activation. In fact a significant redistribution of loads occurs between the connected muscles within a parallel duplex system. The above-mentioned feedback can therefore introduce substantial corrections in the resulting behavior of each individual muscle and of the duplex as a whole. We have developed the basic model used in this study earlier (Izakov et al. 1991; Katsnelson and Markhasin 1996) and it is briefly described in Appendix 2. The above-mentioned feedback mechanisms are allowed for in this model with the help of two types of cooperativity established for contractile proteins.

Numerical Experiments

The duplex modeling program was implemented on an IBM PC using CI language. This program allows the variation of the mechanical characteristics of each of the "muscles" in the duplex model by setting specific numerical values for its parameter (Appendix 2, 3), such as: the series elasticity (β_1), a force scaling factor, which can be interpreted as the thickness of the fiber (λ), the slope and the duration of the ascending limb of the calcium transient (a_c, t_d), the rate constant of calcium binding by troponin (c_1), the slope of the length-force relationship (this slope we varied choosing different values of parameter μ responsible for the end-to-end cooperativity of CaTn), the delay in the excitation of one "muscle" in the duplex relative to the other (Δ).

One "muscle" served as a reference corresponding to a set of basic values for the parameters. The value of one or several of the parameters was then varied in the other "muscle". Thus, in contrast to *in vitro* experiments, the model makes it possible to attribute differences in mechanical characteristics between the "muscles" of a duplex to variations in specific features of given intracellular systems.

In numerical experiments, we simulated duplexes composed of various asynchronously contracting "muscles". In both numerical and physiological experiments, an interaction between muscles undergoing isotonic and physiologic contraction protocols was investigated. The following mechanical characteristics were recorded: the time course of isometric contractions in each "muscle", the length-force and force-velocity relationships, and the time course of relaxation. We also calculated the kinetics of free intracellular Ca^{2+} and the time course of the activation of the contractile proteins by Ca^{2+}(due to the formation of CaTn complexes) in each of the "muscles" when connected in a non-uniform duplex.

Results

Physiological experiments

To demonstrate the effect of inhomogeneity in the duplex system experimentally, we first recorded the contractions of each of the two muscles under various constant loads. We then recorded contractions under similar relative loads of the entire duplex composed of these muscles, and the respective forces developed by the muscles during their interaction in the duplex. In the duplexes, the muscles contracted auxotonically, i.e. under variable loads. The 36 duplexes investigated displayed a variety of mechanical characteristics. A group of duplexes was recognized where the muscle displayed typical individual behavior.

An experimental recording of mechanical activity in a duplex and its muscles using a physiological sequence of mechanical loads illustrates the effect of changes

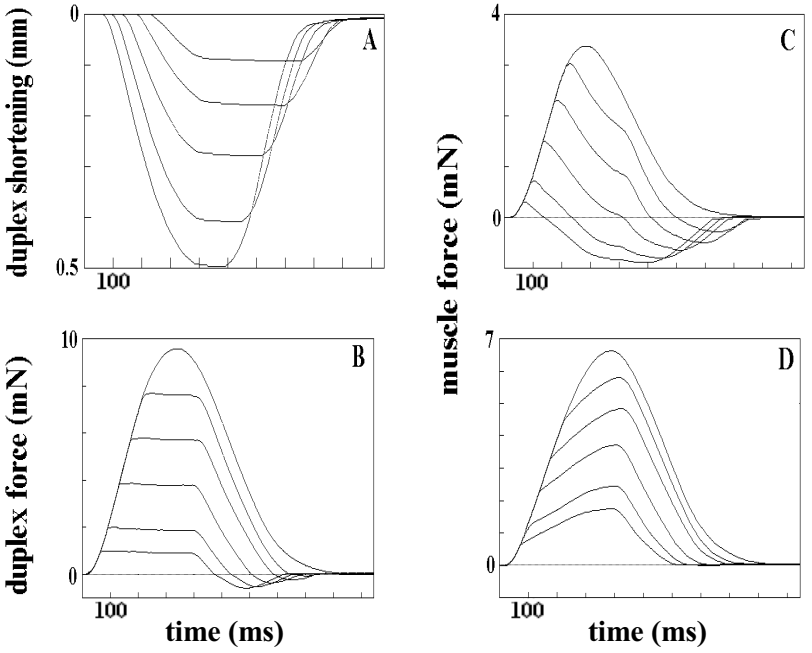

Figure 2. Experimental record of mechanical activity in a duplex composed of two muscles connected in parallel under a physiological sequence of loads: **A.** shortening under the given relative afterload; **B.** force developed by the duplex; **C.** force developed by the first muscle of the duplex; **D.** force developed by the second muscle of the duplex. Mechanical properties of the first muscle and second muscle are as follows. $L_{\max}$: 4.0 mm, 5.0 mm; F_0 – maximal isometric force: 3.4 mN, 6.86 mN; TPF – time to peak force: 246 ms, 346 ms; ΔL – the maximum shortening of a muscle under a relative load equal to $0.1\,F_0$: 0.44 mm, 0.79 mm; $V_{\max}$ – the maximal velocity of shortening of a muscle under a relative load equal to $0.1\,F_0$: 2.85 mm/s, 3.93 mm/s, respectively.

in load (Fig. 2). The mechanical properties of the muscles forming this duplex were quite different. Fig. 2B shows the force development of this duplex undergoing isometric contraction at different relative afterloads (0.1 to 0.8 F_0). Highlighted here are the effects of changes in load on the first (Fig. 2C) and second (Fig. 2D) muscles of the duplex pair. During shortening under a constant load on the duplex, a steep decline in the force of the first muscle and an increase in the force of the second muscle are observed. During isometric relaxation both muscles of the duplex begin to relax simultaneously. Note that in this experiment the force of the parallel elastic element was not subtracted. This explains the occurrence of a negative force in the first muscle. In all the other experiments in this paper the force of the parallel elastic element has been subtracted.

The redistribution of loads on muscles when they contract as a duplex can be of a complex, polyphasic character (Fig. 3). Under a constant load on the duplex,

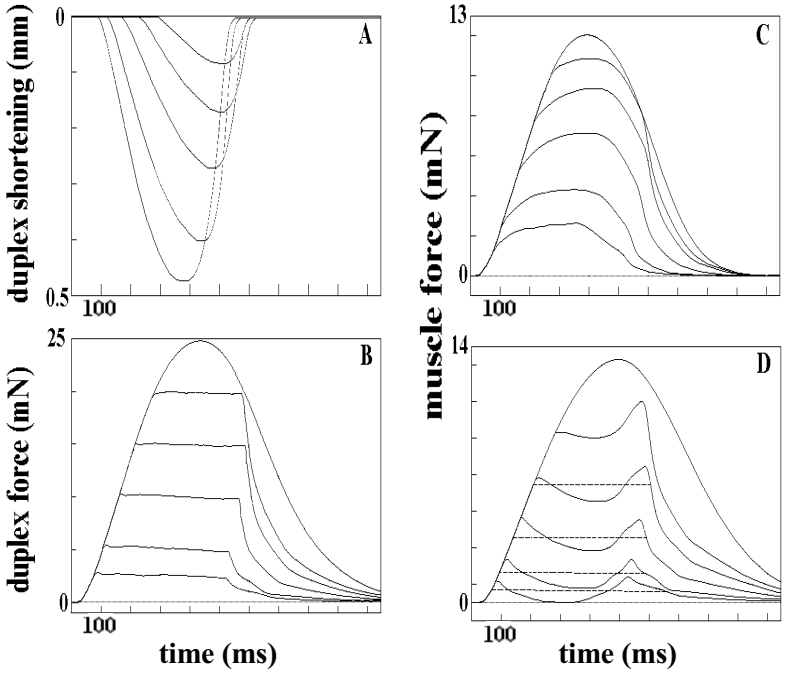

Figure 3. Experimental record of mechanical activity in a duplex composed of two muscles connected in parallel undergoing isotonic contraction: **A.** shortening under the given relative afterload; **B.** force developed by the duplex; **C.** force developed by the first muscle of the duplex; **D.** force developed by the second muscle of the duplex. Mechanical properties of the first muscle and second muscle are as follows. $L_{\max}$: 5.0 mm, 4.0 mm; F_0: 12.0 mN, 13.24 mN; TPF: 364 ms, 450 ms; ΔL: 0.58 mm, 0.40 mm; $V_{\max}$: 3.93 mm/s, 1.72 mm/s, respectively. Dashed traces indicate average values of force to reveal magnitudes of force oscillations.

the increase in force of the first muscle in this duplex is retarded (Fig. 3C). The force of the second muscle is characterized by marked fluctuations relative to the level of the load (as shown in Fig. 3D by the dashed lines).

Some duplex pairs exhibited behavior that was typical of the individual muscles that composed them, i.e. each muscle in a duplex contracted almost isotonically. Such a redistribution of mechanical loads is characteristic for duplexes consisting of muscles demonstrating similar mechanical properties such as the amplitude of isometric contractions, TPF, and maximum shortening velocities.

Figs. 2 and 3 apply to the cases where the muscles were excited simultaneously, although they had different time courses of force development, i.e. were asynchronous. Non-simultaneous excitation of the muscles in a duplex pair was used as the other method of composing asynchronous duplexes. A group of experiments designed to study the effects of asynchronism in the duplex on the mechanical characteristics of the myocardium, showed a substantial effect of asynchronism on the distribution of loads in the muscles of a duplex pair.

An example of an initial asynchronous duplex (muscles had different TPF of isometric contractions) is shown in Fig. 4. During a contraction of this duplex, the force of the first muscle was observed to fall off monotonously. Growth in the force of the second muscle demonstrates a biphasic pattern at large afterloads. During the isometric phase of duplex relaxation the force of the second muscle continues to grow.

By delaying the excitation of the first muscle relative to the second by 130 ms we obtained a duplex exhibiting coincidence of the peaks of the isometric contractions of the individual muscles (Fig. 5). This led to a sharp change in the redistribution of loads in the duplex. The isometric contraction of this duplex displays a gentle increase in force for 200 ms followed by 250 ms of steeper growth (Fig. 5B). During the isotonic phase of the duplex shortening and prior to the coincidence of peaks, the first muscle declined in tension (Fig. 5C) and the second displayed a polyphasic increase in force (Fig. 5D). Following the peak of isometric contraction the pattern reversed. The force of the first muscle was observed to increase, while that of the second showed a polyphasic decrease. The character of this decrease, however, is different from that which occurred before the coincidence of peaks. During isometric relaxation, the force of contraction of the second muscle continues to increase at large afterloads. A pattern of load redistribution similar to that observed in the initially asynchronous duplex (Fig. 4) is obtained by delaying the excitation of the second muscle relative to the first by 100 ms, but accompanied by a steeper decline in the force of the first muscle and a steeper growth in the force of the second one than in the previous example.

Numerical experiments

In the previous examples we presented the results of the physiological experiments on inhomogeneous muscle duplexes. In order to understand how the mechanical

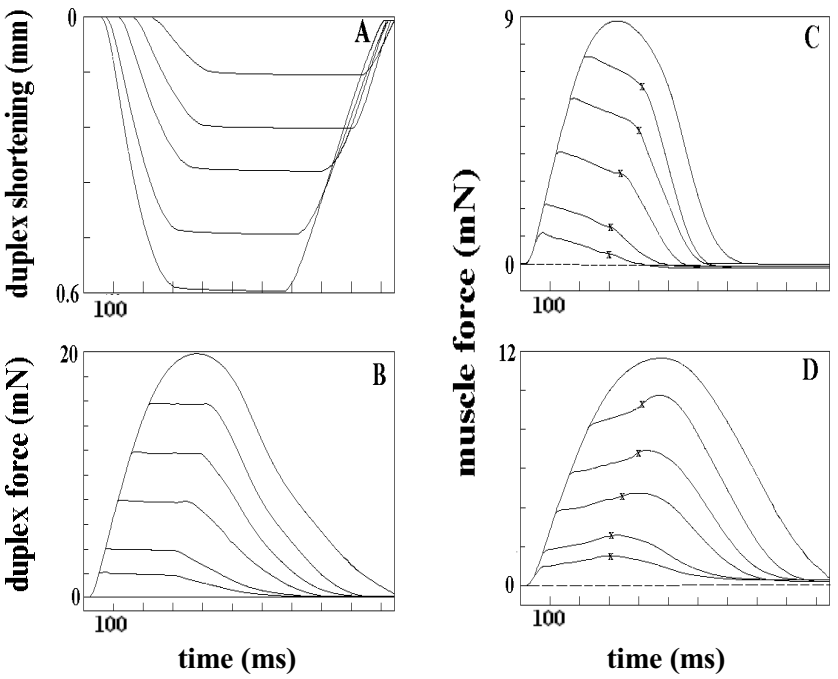

Figure 4. Experimental record of mechanical activity in an initially asynchronous duplex (asynchronism – 130 ms) composed of two muscles connected in parallel under a physiological sequence of loads: **A.** shortening under the given relative afterload; **B.** force developed by the duplex; **C.** force developed by the first muscle of the duplex; **D.** force developed by the second muscle of the duplex. Mechanical properties of the first muscle and second muscle are as follows. $L_{\max}$: 4.0 mm, 4.2 mm; F_0: 8.87 mN, 11.67 mN; TPF: 300 ms, 430 ms; ΔL: 0.52 mm, 0.66 mm; $V_{\max}$: 3.93 mm/s, 3.93 mm/s, respectively.
x indicates the end of the afterloaded phase of a duplex contraction.

behavior of the muscles of a duplex can be dependent on the internal parameters of the system, we used a mathematical model within which inhomogeneity could be set by varying the parameters controlling the contractile action of each of the muscles. In this work, we used duplexes composed of three simulated "muscles". Two of them ("muscle 2" and "muscle 3") were obtained from "muscle 1", characterized by a basic set of parameters, by changing certain parameters of the model (Appendix 3).

Fig. 6 shows the results of a representative numerical experiment on a duplex consisting of "muscles" 2 and 1. The value $L_{\max} = 2.24$ μm/sarcomere was chosen for the model as $L_{\max}$ of the "muscle" according to laser diffraction data. All lengths in the numerical model are related to the sarcomere, i.e. the current length, the end-systolic length and the shortening of a fiber are expressed as the current length, the end-systolic length and the shortening of the sarcomere of this fiber.

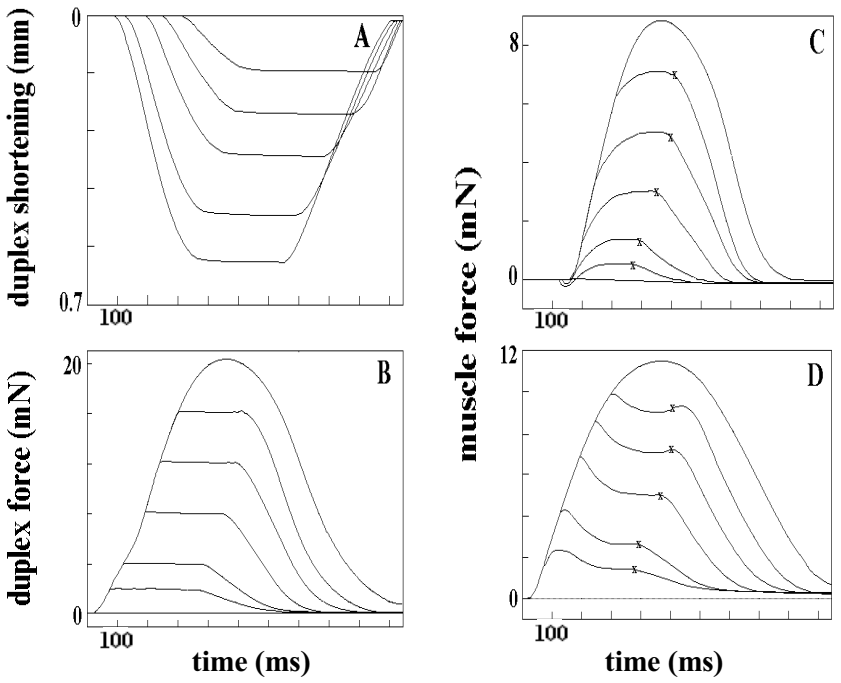

Figure 5. Experimental record of mechanical activity in a duplex obtained by delaying the excitation of the first muscle with respect to the other by 130 ms (coincidence of peak isometric contractions of the muscles): **A.** shortening under the given relative afterload; **B.** force developed by the duplex; **C.** force developed by the first muscle of the duplex; **D.** force developed by the second muscle of the duplex.
x indicates the moment of the end of afterloaded phase of the respective duplex contraction.

Figs. 6A and 6B show the *in vitro* shortening of the duplex under different relative afterloads (0.1 to 0.9 F_0), and the simulated development of force by a numerical duplex undergoing isometric contraction. Fig. 6 illustrates the pattern of change in the load in the first (Fig. 6C) and second (Fig. 6D) "muscle" during a contraction of the duplex. This duplex is characterized by the redistribution of loads in it so that the force of the first "muscle" is observed to decline during the isotonic phase (Fig. 6C) while that of the second "muscle" increases (Fig. 6D) over the entire range of duplex shortenings. Note that the relationship between the mechanical characteristics of the "muscles" forming this simulated duplex is in agreement with the physiological experiment shown in Fig. 2.

Fig. 7 shows a marked polyphasic pattern of changes in the force of the "muscles" contracting within the duplex. Under a constant load on the duplex, the increase in force of the first "muscle" is replaced by a decline in force (Fig. 7C). The

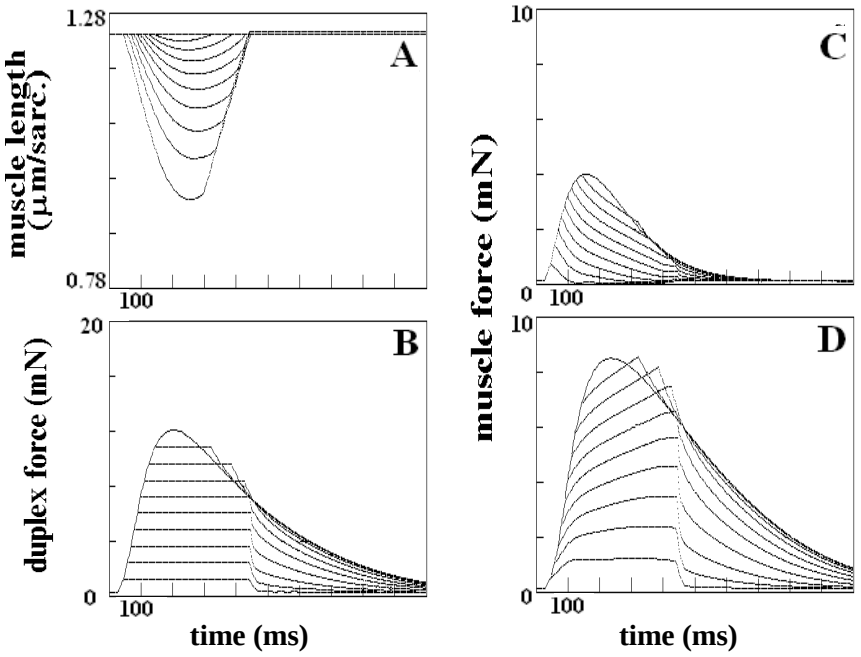

Figure 6. Results of numerical experiment for the duplex model: **A.** changes in the length of the "muscle" during shortening; **B.** force developed by the duplex; **C.** force developed by the first "muscle" of the duplex; **D.** force developed by the second "muscle" of the duplex. Mechanical properties of the first "muscle" and second "muscle" are as follows. L_{max}: 2.24 μm/sarcomere, 2.24 μm/sarcomere; F_0: 4 mN, 8.55 mN; TPF: 155 ms, 235 ms; ΔL: 0.18 μm/sarcomere, 0.34 μm/sarcomere; V_{max}: 0.41 μm/s, 0.68 μm/s, respectively. V_{max} is the maximum shortening velocity of the muscle at a given afterload in fractions of V_m (V_m is a model parameter equal to 2.5 muscle length/s, which sets a certain maximum-velocity limit).

force of the second "muscle" demonstrates a marked polyphasic pattern (Fig. 7D). The initial increase in the force is replaced by a decline, followed by a subsequent steep increase. A polyphasic pattern, similar to that observed in the numerical experiment, was observed in the physiological experiment as well (Fig. 3). However, in this numerical experiment we failed to obtain the same relationship between the mechanical characteristics of the "muscles" of the duplex as in the physiological experiment.

Numerical experiments on duplex models formed by delaying the excitation of one "muscle" relative to the other have confirmed the effect of asynchronism on the pattern of load redistribution in each of the duplex "muscles". Moreover, the mathematical model enables us (in contrast to physiological experiments) to study the effect of asynchronism in "pure" form. Having obtained an ideal uniform

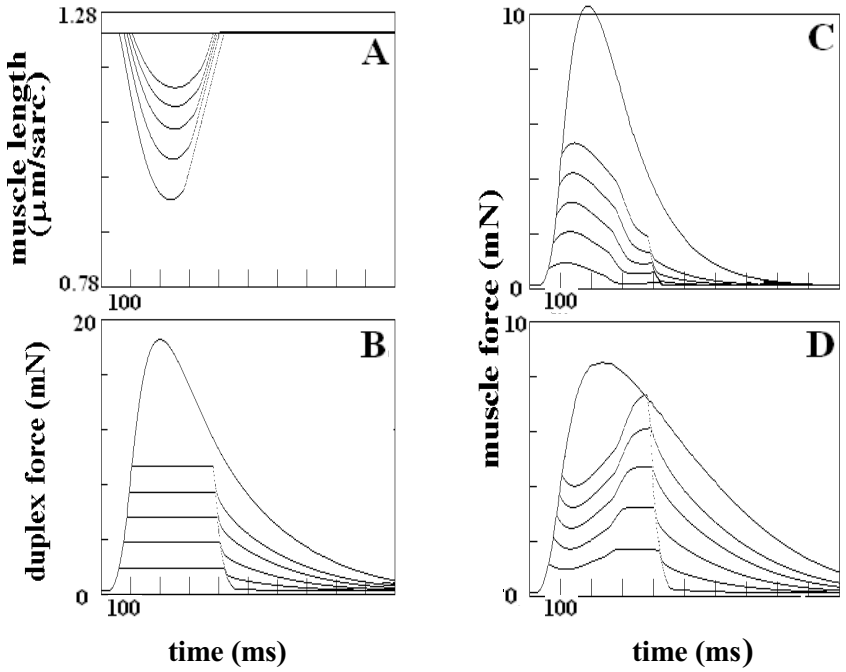

Figure 7. Results of numerical experiment for the duplex model. **A.** changes in the length of the "muscle" during shortening; **B.** force developed by the duplex; **C.** force developed by the first "muscle" of the duplex; **D.** force developed by the second "muscle" of the duplex. $L_{\max}$: 2.24 μm/sarcomere, 2.24 μm/sarcomere; F_0: 10.2 mN, 8.55 mN; TPF: 185 ms, 235 ms; ΔL: 0.27 μm/sarcomere, 0.34 μm/sarcomere; $V_{\max}$: 0.75 μm/s, 0.68 μm/s, respectively.

duplex, i.e. one in which all the mechanical characteristics of the "muscles" are identical, it would be possible to investigate the effects attributed to asynchronism only by changing the time of the delay in excitation between the "muscles". In physiological experiments this is practically impossible because of differences in mechanical characteristics between the muscles.

Fig. 8 shows the results of a numerical experiment on the model of a duplex formed by delaying the excitation between "identical muscles" by 100 ms. The administration of a delay in the excitation of the first "muscle" relative to the other changed the pattern of the redistribution of loads between the "muscles". Increased force of the first "muscle" (Fig. 8C) and a decrease in the force of the second (Fig. 8D) replaced a constant load on the "muscles".

The results of the numerical experiment were also obtained simulating the data of physiological ones shown in Fig. 5. The excitation of the first "muscle" was compared relative to the second one from an initially asynchronous duplex shown

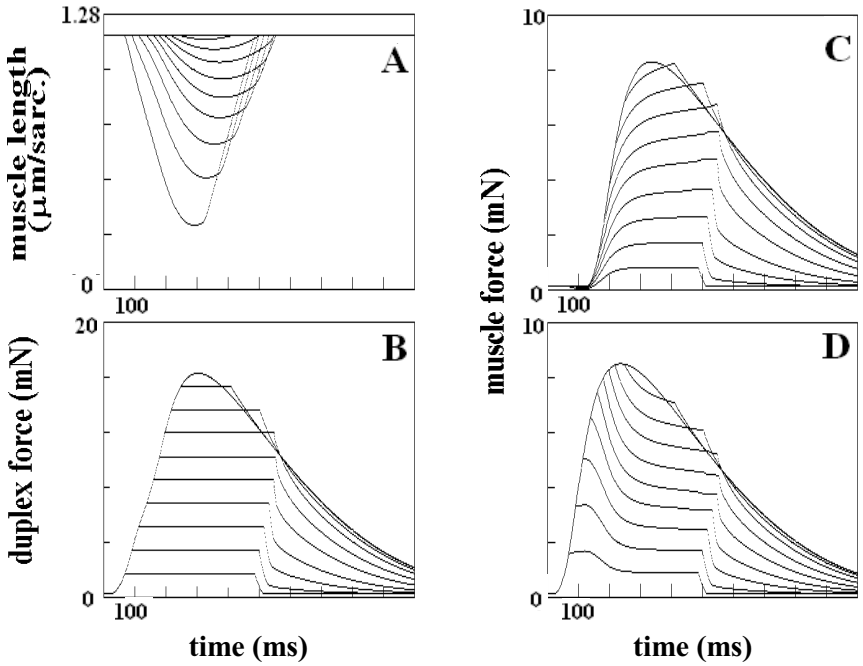

Figure 8. Results of numerical experiment on the model of a duplex formed by delaying the excitation of the first "muscle" relative to the second one by 100 ms in a uniform duplex: **A.** changes in the length of the "muscle" during shortening; **B.** force developed by the duplex; **C.** force developed by the first "muscle" of the duplex; **D.** force developed by the second "muscle" of the duplex.

in Fig. 6. Specifically, that was done to achieve coincidence of the peaks of the isometric contractions of both "muscles". The same patterns of the changed time-course of the muscle force as revealed in Fig. 5 for the physiological experiment were observed in the numerical one as well.

Mathematical modeling allows us to evaluate changes in the kinetics and the level of free intracellular calcium in cardiomyocytes. In the model it is also possible to trace the formation of CaTn complexes (contractile proteins activation process) in each of the "muscles" in isolation and within a duplex.

Figs. 9 and 10 present the results of a numerical experiment which show that the connection of "muscles" in a duplex and mechanical interaction between them result in a change in the time course, in the free calcium content, and in CaTn complexes in each of the "muscles". We present an illustration of this statement for the pairs of "muscles" that are shown in Fig. 6. It can be seen that the first (weak) "muscle" of the duplex demonstrates a great change in the time course of the level of free calcium at small afterloads, whereas at large afterloads the kinetics

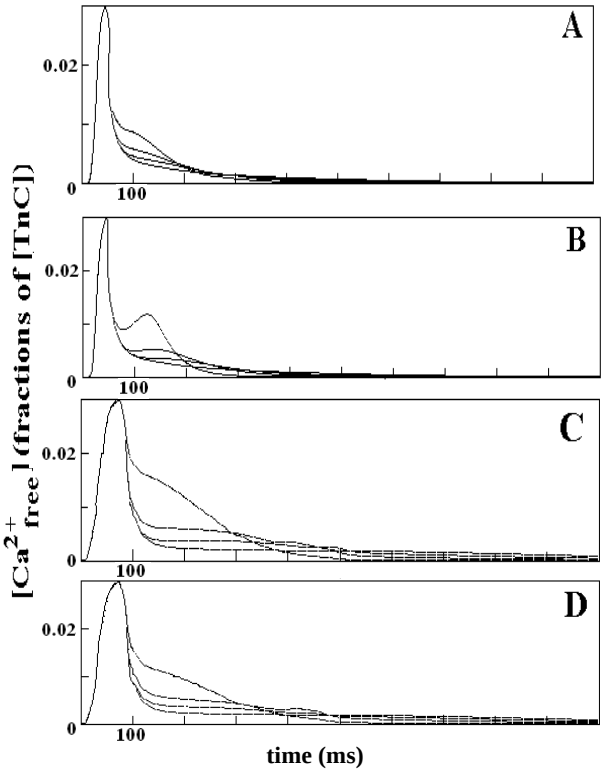

Figure 9. Time course of free intracellular Ca^{2+}: **A.** for the first "muscle" contracting in isolation; **B.** for the same "muscle" within a duplex; **C.** for the second "muscle" contracting in isolation; **D.** for the second "muscle" within a duplex. The curves are arranged from top to bottom in the increasing order of afterloads ($0.1\,F_0$, $0.3\,F_0$, $0.5\,F_0$ and F_0).

of the free calcium practically does not change. At the same time, in the second (stronger) "muscle" the time course of the level of free calcium is observed to change at all afterloads. In the same way, the time course of activation is different in the second "muscle" for all afterloads whereas in the first "muscle" differences are evident only at small afterloads.

Discussion

Mechanical inhomogeneity of the myocardium is a well-established phenomenon. However, the mechanical laws which determine the contribution of mechanical inhomogeneity to the pumping and contractile function of the heart remain unclear. Study of this phenomenon in an intact heart is a complex problem: mechanical interaction between various parts of the heart is influenced by its geometry, specific

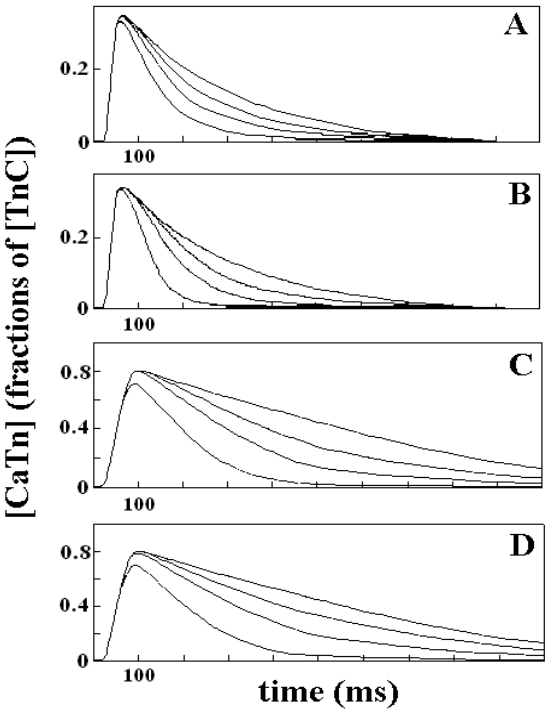

Figure 10. Changes in the concentration of Ca^{2+}-troponin complexes with time: **A.** for the first "muscle" contracting in isolation; **B.** for the same "muscle" within a duplex; **C.** for the second "muscle" contracting in isolation; **D.** for the second "muscle" within a duplex. The curves are arranged from top to bottom in the increasing order of afterloads ($0.1\,F_0$, $0.3\,F_0$, $0.5\,F_0$ and F_0).

structure of muscle fibers in the heart chamber walls and neurohumoral effects. In order to gain a better understanding of this complex phenomenon, mechanical interactions between the contractile elements of myocardial tissue have to be separated from such factors.

An elementary, mechanically non-uniform system representing myocardial tissue should consist of two mechanically different elements connected in series or in parallel. This non-uniform system should provide information about the individual mechanical characteristics of each of the elements in the system, how they are modified when combined in a non-uniform system, and how each influences the system as a whole.

A muscle duplex was first studied by Tyberg et al. Unlike us, these authors focused on muscles connected in series rather than in parallel. In particular, they used papillary muscles excised from cat right ventricle. Both the duplex and one of its muscles (muscle I) in isolation were exposed to a series of after-loaded contractions. Force-velocity relationships were established for both the duplex and muscle I by plotting peak velocity of duplex (or muscle) shortening under each afterload *vs.* this afterload value. Then they assessed force-velocity relationship for the other muscle (II) of the duplex subtracting corresponding relationship of muscle I from

that of the whole duplex. During the experiments one of the muscles was exposed to various local influences (e.g. hypoxia or/and different delays of the muscle's excitation). It was a pioneering work showing experimentally how either inhomogeneity of a duplex may affect its mechanical function.

However, this approach is actually not appropriate for clarification of the real effect of the mechanical interaction on the behavior of the duplex elements as the above assessment of the force-velocity relationship of muscle II was incorrect. First, the authors did not take into account length redistribution between the muscles (i.e. just the interaction) during isometric phase of contraction before the isotonic one. This interaction brought muscle I to have a different length at the beginning of isotonic shortening as compared to its isolated contraction under the same afterload, and thus changed both the time of the peak velocity of the muscle's shortening and the value of this peak as such. Therefore, calculating force-velocity relationship of muscle II within the duplex, the authors subtracted non-actual values of peak shortening velocities of the first muscle. Second, the procedure of subtraction was incorrect by itself. Indeed, series connection could not synchronize time to peak shortening velocity in both muscles (unlike the parallel connection), i.e. these peaks were attained by each muscle and by the whole duplex at different moments.

Unlike this approach the method proposed here allows us to account for real contribution of interaction between duplex elements, because we record mechanical characteristics of two muscles (force-velocity and length-force included) when they contract both in isolation and within the duplex.

Wiegner et al. (1978) simulated a duplex consisting of a hypoxic and a normal myocardium connected in series. First the computer memorised the isotonic contractions of the muscle under normal conditions. This muscle was then subjected to hypoxia and was made to contract under load applied by the computer which remembered the isotonic contractions of the normal muscle. In this case, obviously, there was no feedback between the normal and the hypoxic muscle which constitutes the essence of the interaction between inhomogeneous elements in the myocardium.

We believe that our experimental methods utilizing muscle duplexes and their mathematical modeling provides insights with reference to several basic aspects of the complex myocardial systems at the tissue level. A redistribution of tensions, which sometimes may be very complicated (polyphasic), takes place in the elements of an elementary non-uniform myocardial system (a duplex with muscles connected in parallel) at the tissue level when it is shortened under a constant load; this complex redistribution depends on the degree of difference between the mechanical characteristics of the system elements and on the afterload on the duplex. For given end-systolic lengths and tensions the elements of the duplex may fail to develop end-systolic tensions and the timing of the onset of isometric relaxation in the duplex elements may not coincide with the beginning of the isometric relaxation

phase of the duplex. Asynchronism in the excitation of the elements in a duplex has a substantial effect on the redistribution of tensions in them, which critically depends on the excitation sequence. Mathematical modeling demonstrates that mechanical inhomogeneity can cause a significant effect on the Ca^{2+} transient, the level and the time course of CaTn complex formation in the cells of the elements of a non-uniform myocardial system. Moreover, an oppositely directed activation of the thin filament by Ca^{2+} in the elements of a duplex has been found in the model: an increase in one element is accompanied by a decrease in the other.

Thus, the principal outcome of this work is experimental evidence that in a non-uniform myocardial system (duplex) with muscle elements connected in parallel, contraction of the duplex under a constant load produces, as the main event, a redistribution of mechanical tensions in the elements depending first and foremost on magnitude and character of asynchronism.

This redistribution of tensions within the elements of a non-uniform myocardial system is important because it can influence the fundamental mechanical characteristics of the individual elements. Mechanical characteristics of the myocardium such as length-force (Brady 1967), force-velocity (Edman 1977) and end-systolic length-relaxation time relationships (Brutsaert and Sys 1989) are directly dependent on mechanical conditions under which these characteristics are recorded. Thus, the slope of the length-force relationship is steeper during the isotonic protocol as compared with the isometric one (Brady 1967). The steepness of the force-velocity relationship depends on whether it is recorded with reference to afterload contractions or by the method of fast release (Edman 1977). For the same level of mechanical tension the time constant of isometric relaxation is greater than one obtained for the isometric phase following an isotonic contraction. Moreover, the isometric phase of isotonic relaxation varies depending on whether the load on the muscle is altered during the early or the late phase of isotonic contraction (Brutsaert and Sys 1989).

All of the mechanical phenomena demonstrated by *in vitro* experimentation support the idea that Ca^{2+} activation of the thin filament depends on the mechanical conditions of the myocardium (Allen and Kentish 1985). For example, the time course of the Ca^{2+} transient differs for isometric and isotonic contractions (Housmans et al. 1983). These results are supported by mathematical models (Panerai 1980; Izakov et al. 1991; Peterson et al. 1991; Landesberd and Sideman 1994) explaining a wide range of mechanical phenomena in the myocardium on the basis of a close feedback between mechanical conditions of myocardial contraction and the activation of contractile proteins by Ca^{2+}. Our mathematical model (which includes feedback control) was able to reproduce experimental data successfully. The results of numerical modeling show that one of major mechanisms which underlie the influences of mechanical inhomogeneity on the contractile function of the myocardium is the change in the processes of activation of the thin filaments. The model provides evidence of a compensatory charac-

ter of these changes since these changes in the elements are opposite in character.

For the duplex as a whole these changes are compensating in character (Markhasin et al. 1997a) though, in general, compensation is partial only. For the duplex as a whole any inhomogeneity manifests itself as either a negative or a positive inotropic effect. The force potential of the duplex established by the dynamic length-force relationship is not an additive function of the force potentials of its interacting elements. This conclusion is valid for both parallel and serial connections of the elements. In the former, however, the main factor of non-additivity is the redistribution of tensions in the elements of the duplex, while in the latter it is the redistribution of length (Markhasin et al. 1997a).

In addition to changes in thin filament activation during an interaction, numerical analysis allowed us to find other mechanisms responsible for the experimentally observed effects. At certain relative afterloads on the elements within a duplex their end-systolic lengths and the time to achive these lengths differ from those recorded when the elements contracted individually. Thus, when the elements are interacting within a duplex, the end-systolic length and force are achieved at different degrees of overlap between the thick and thin filaments and at different levels of Ca^{2+} activation than when those elements contract in isolation.

Our experiments and numerical tests revealed that the main determinant of myocardial inhomogeneity is asynchrony. Asynchrony can manifest itself either as a different time course of contractions in the simultaneously excited elements of a non-uniform system (Figs. 2, 3, 4) or as different time delays in the excitation of uniform elements relative to each other, or as a combination of these events (Fig. 5). An important property of asynchrony as established in this work is the non-commutativity of its influence so that the redistribution of tensions in the elements of a contracting duplex critically depends on the excitation sequence.

Based on the present results and on the well-known close relationship between mechanical conditions of myocardium contraction and the process of Ca^{2+} activation of contractile proteins, we suggest the following general hypothesis: because mechanical interaction between non-uniform elements of the myocardium permanently changes mechanical conditions for each element, mechanical inhomogeneity should produce a substantial effect on the ino-, lusi- and ergotropic functions of cardiac muscle. This, however, is a subject for another study.

Our model of a parallel muscle duplex also simulates an interaction between the parallel layers of the cardiac muscle wall. In a non-uniform duplex, one of the muscles is weaker. As it has been shown, a shortening under a constant load results in a redistribution of tensions in the elements of the duplex whereby the larger share of the total load falls on the stronger muscle. Such a situation is probable in pathologic states, such as myocardial ischemia. The weaker (conventionally ischemic) layer becomes unloaded, which may contribute to its survival. The stronger (conventionally normal) layer is overloaded and clearly the energy status of this

layer critically depends on the conditions of blood circulation. If microcirculation in this layer is normal and the oxygen supply is adequate to the work being done, the cumulative effect of the interaction between the two layers should be positive, facilitating the survival of the cardiac muscle. If there is an insufficient supply of oxygen to the overstrained layer, the ischemic zone may expand. It is clear that the final outcome of interaction between the affected and normal layers corresponds to a dynamic balance between the extent of damage and the microcirculatory reserve.

Landesberg et al. (1996) made an attempt, using a mathematical model for a duplex with its elements connected in parallel, to clarify the mechanism underlying mosaic damage to cardiomyocytes (i.e. the presence of normal cells alongside damaged ones) in the case of subendocardial infarction in the myocardium. It was shown that during the interaction between the ischemic subendocardial layer of the myocardium and the normal subepicardial one, the normal layer accelerates the reduction of the ischemic layer and leads to a decrease in its oxygen demand. The following dependence was also found out: the smaller the surviving area size the higher the acceleration effect.

The oppositely directed redistribution of tensions in the parallel interacting elements of the duplex and variously directed activation of the thin filament indicate that non-uniform myocardial systems compensate inhomogeneity effects. Thus, data and their analysis with the help of a mathematical model show that, (1), it is not enough to establish the fact of segmental inhomogeneity in the heart chamber walls. Knowledge is required of the mechanical characteristics of each of the segments and their local perfusion, (2), in pathologic states it is important to estimate not only the global pumping and contractile functions of the heart but also the local mechanical status of the segments: in view of the compensatory character of inhomogeneity effects the global function can change but slightly whereas locally a given segment can become a source of damage.

Acknowledgements. At first, this work was supported by RFBR # 96-04-50041, CRDF RN1-420, and then by RFBR # 00-04-48323, and Wellcome Trust Grant # 061115/Z/00/Z.

Appendix 1

Mathematical model for muscle duplexes

Parallel muscle duplexes are simulated using the basic model of a single fiber, i.e. a system of equations describing individually each of the elements combined in a duplex. In so doing each of the elements is characterized by its own set of values for the parameters of the model, while their interaction is given by determining relationships added to the main equations describing the properties of interaction between the elements.

For a parallel connection the relationships added are given by:

(i) $L_1 = L_2 =$ constant for the isometric contraction protocol

(ii) $L_1 = L_2$, $P_1 + P_2 = D =$ constant for the isotonic contraction protocol,

where P_1, L_1 are the tensions and the lengths of the first muscle, P_2, L_2 – those of the second muscle. D is the afterload. In a similar way, the model describes physiological contraction for a duplex.

Appendix 2

The equations of the mathematical model for contractions of a homogeneous fiber

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

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

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

$$\dot{Ca}_{\mathrm{f}} = -\dot{A}_1 - \dot{B} - r(Ca_{\mathrm{f}}) \cdot Ca_{\mathrm{f}} \quad (4)$$

$$\dot{B} = b_{\mathrm{on}} \cdot (B_s - B) \cdot Ca_{\mathrm{f}} - b_{\mathrm{off}} \cdot B \quad (5)$$

The phase variables of the set of equations have the following meaning: l_1 is the deviation in the length of the contractile element from the length at rest; A_1 is the average concentration of Ca^{2+}-troponin complexes in the overlap between the thin and thick filaments; n_2 is the average probability that a myosin cross bridge will attach to a free actin site it "finds" on the thin filament; l_2 is the deviation in the length of the muscle from the length at rest. Ca_{f} is the concentration of free intracellular calcium. For $t \leq t_{\mathrm{d}}$ (t_{d} is the time to peak calcium transient) Ca_{f} is given by the equality $Ca_{\mathrm{f}} = Ca(t)$, where $Ca(t)$ is an explicit function. But for $t \geq t_{\mathrm{d}}$ Ca_{f} is described by differential equation (4) with the initial condition: $Ca_{\mathrm{f}}(t_{\mathrm{d}}) = Ca(t_d)$. B is the concentration of Ca^{2+}-buffer complexes. B_{s} is the capacity of the buffer expressed, like all the other concentrations, in fractions of the concentration of TnC in the cell. Also, the equations contain the following dependencies, in the model given in an explicit form:

$$p(\dot{l}_1),\ Ca(t), n_1(l_1),\ \Pi(n_1(l_1) \cdot n_2),\ q_n(\dot{l}_1),\ G^*(\dot{l}_1),\ r(Ca_{\mathrm{f}})$$

where p is the average force developed by a cross bridge (p is expressed in relative units, it equals 1 for $\dot{l}_1 = 0$); n_1 is the average probability that a cross bridge will "find" a free actin site on the thin filament. For our detailed substantiation of the length dependence of probability n_1 see Izakov et al. 1991. In addition to n_1, length-dependence is displayed by quantity $\Pi(n_1(l_1) \cdot n_2)$ as well, which is the variable part of the rate constant of Ca^{2+}-troponin complexes disintegration specifying the first type of cooperative dependence of this constant on the concentration of cross bridges attached to the filament. $q_n = q_n(\dot{l}_1)$ and $G^* = G^*(\dot{l}_1)$ are functional dependencies relating the kinetics of change in quantity n_2 to the shortening ve-

locity of the contractile element. $\bar{n}_2$ is the model parameter meaning an average probability that a cross bridge will find a free actin site on the thin filament in specific conditions of the absolute isometry, i.e. when sarcomeres have a permanently constant length. Term $r(Ca_\mathrm{f}) \cdot Ca_\mathrm{f}$ in equation (4) stands for the uptake of free intracellular Ca^{2+} by the sarcoplasmic reticulum (SR). With the help of term $r(Ca_\mathrm{f})$ we describe the uptake function of the SR. In particular, it is used to set the negative feedback: the higher the concentration of intracellular free Ca^{2+}, the stronger the repression of the pump taking up this Ca^{2+} in SR. Hence, function $r(Ca_\mathrm{f})$ should meet the requirement of strong convergence to zero with increasing argument Ca_f. In the model, therefore, we set: $r(Ca_f) = r_\mathrm{Ca} \cdot \exp(-q_\mathrm{Ca} \cdot Ca_\mathrm{f})$, where r_Ca and q_Ca are parameters of the model.

The quantities not mentioned in the above list but appearing in the equations are constant parameters of the model. We omit the deduction of the above equations as well as formulas specifying the dependencies in an explicit form: $p(\dot{l}_1), Ca(t), n_1(l_1), \Pi(n_1(\dot{l}_1) \cdot n_2), G^*(\dot{l}_1)$ and $q_n(l_1)$. Note only that function $p(\dot{l}_1)$ is reversible in an explicit form; this quality makes it possible to solve the first equation of the set for $\dot{l}_1$. This leads to the Cauchy form of the equations (allowing for easily established initial values) for phase variables l_1, A_1, n_2. The force developed by the contractile element is given in the model by the formula: $P_\mathrm{CE} = \lambda \cdot p(\dot{l}_1) \cdot A_1^\mu \cdot n_2 \cdot n_1(l_1) \cdot (l_1 + S_0)$ The first equation of the above set follows from equality: $P_\mathrm{CE} = P_\mathrm{SE}$, where $P_\mathrm{SE} = \beta_1 \cdot [\exp(\alpha_1 \cdot (l_2 - l_1)) - 1]$ is the force of the series elastic element. The force of the muscle is equal to $P_\mathrm{CE} + P_\mathrm{PE}$, where P_PE is the force of the parallel elastic element given by: $P_\mathrm{PE} = \beta_2 \cdot [\exp(\alpha_2 \cdot l_2) - 1]$.

We have already emphasized that the main feature of the previous model was feedback in the kinetics of Ca^{2+}-troponin complexes described by the second equation of the model. This feedback implemented in two types of cooperativity in contractile proteins is reflected in the constant for the disintegration of Ca^{2+}-troponin complexes: $c_{20} \cdot \exp(-q_k \cdot A_1) \cdot \Pi(n_1(l_1) \cdot n_2)$. The first multiplier c_{20} is a parameter of the model. Term $\Pi(n_1(l_1) \cdot n_2)$ stands for cooperativity of the first type: the dependence of the kinetics of Ca^{2+}-troponin complexes along the thin filament. Term $\exp(-q_k \cdot A_1)$ sets cooperativity of the second type: interaction between Ca^{2+}-troponin complexes along the thin filament leading to a slow-down in their dissociation. This other type of cooperativity (namely the so called end-to-end cooperativity of CaTn) is also used in the model (see A^μ ($\mu \geq 1$) in both equation (1) and above-given formula for P_CE). We justified in detail this cooperativity elsewhere (Katsnelson and Markhasin 1996). In short its sense is as follows. Physiologically the variant of $\mu > 1$ means direct interrelation between calcium-troponin complexes (not through kinetic constants) enhancing the ability of each of them to derepress sites on the actin and, hence, increasing the contribution of each of them to the development of muscle tension. Thus, the number of derepressed actin sites turns out to be proportional to a degree of A (where A is [TnC]) rather then to A as itself.

Appendix 3

Basic parameters of the mathematical model for a homogeneous fiber

The set of the parameter values we used in our numerical experiments are taken from the published variant of our homogeneous muscle model (Izakov et al. 1991). It is as follows:

$\alpha_1 = 14.6\ \mu\text{m}^{-1}$	$\lambda = 20\ \text{g}\cdot\text{mm}^{-2}\cdot\mu\text{m}^{-1}$	$q_{\text{Ca}} = 50$
$\beta_1 = 0.56\ \text{g}\cdot\text{mm}^{-2}$	$S_0 = 1.14\ \mu\text{m}$	$b_{\text{on}} = 2.0\ \text{ms}^{-1}$
$\alpha_2 = 14.6\ \mu\text{m}^{-1}$	$t_{\text{d}} = 85\ \text{ms}$	$b_{\text{off}} = 0.14\ \text{ms}^{-1}$
$c_1 = 1.6\ \text{ms}^{-1}$	$a = 0.25$	$B_{\text{s}} = 0.4$
$\beta_2 = 0.00012\ \text{g}\cdot\text{mm}^{-2}$	$r_{\text{Ca}} = 0.5$	$\mu = 1.7$
$c_{20} = 0.6\ \text{ms}^{-1}$		$\bar{n}_2 = 0.87$

All concentrations appearing in the model equations are defined in fractions of troponin (TnC) concentration, where $[\text{TnC}] = 7.0 \times 10^{-5}$ mol/l. The parameter values for "muscle 1" were used for control. "Muscle 2" was obtained by altering some parameters of "muscle 1" as follows: $\beta_1 = 1.6\ \text{g}\cdot\text{mm}^{-2}$; $\lambda = 48\ \text{g}\cdot\text{mm}^{-2}\cdot\mu\text{m}^{-1}$; $t_{\text{d}} = 40$ ms; $c_1 = 0.9\ \text{ms}^{-1}$. "Muscle 3" was obtained from "muscle 1" by setting the following: $\beta_1 = 0.33\ \text{g}\cdot\text{mm}^{-2}$; $\lambda = 120\ \text{g}\cdot\text{mm}^{-2}\cdot\mu\text{m}^{-1}$; $\mu = 3$.

References

Allen D. G., Kentish J. C. (1985): The cellular basis of the length-tension relation in cardiac muscle. J. Mol. Cell. Biol. **17**, 821—840

Azhari H., Buchalter M., Sideman S., Shapiro E., Beyar R. (1992): A conical model to describe the nonuniformity of the left ventricular twisting motion. Ann. Biomed. Eng. **20**, 149—165

Beyar R., Sideman S. (1987): A model of coronary perfusion and its control as related to cardiac mechanics. In: Simulation and Control of the Cardiac System (II), pp. 181—197, CRC Press, Inc., Boca Raton, Florida

Brady A. J. (1967): The tree element model of muscle mechanics: its applicability to cardiac muscle. Physiologist. **10**, 75—86

Brutsaert D. L., Sys S. U. (1989): Relaxation and diastole of the heart. Physiol. Rev. **69**, 1228—1315

Edman K. A. (1977): The instantaneous force-velocity relationship as on index of the contractile state in cardiac muscle. Basic Res. Cardiol. **72**, 94—101

Gallagher K. P., Gerren R. A., Stirling M. C. (1986): The distribution of functional impairment across the lateral border of acutely ischemic myocardium. Circ. Res. **58**, 570—583

Haendchen R. V., Wyatt H. L., Maurer G., Zwehl W., Bear M., Meerbaum S., Corday E. (1983): Quantitation of regional cardiac function by two-dimensional echocardiography: I. Patterns of contraction in the normal left ventricle. Circulation **67**, 1234—1245

Hosino T., Fujiwara H., Kawai C., Hamashima Y. (1983): Myocardial fiber diameter and regional disrtibution in the ventricular wall of normal adult hearts, hypertensive hearts and hearts with hypertrophic cardiomyopathy. Circulation **67**, 1109—1116

Housmans P. R., Lee N. K., Blinks J. R. (1983): Active shortening retards the decline of the intracellular calcium transient in mammalian heart muscle. Science (Washington D. C.) **221**, 159—161

Huisman R. M., Sipkema P., Westerhof N., Elzinga G. (1980): Comparison of models used to calculate left ventricular wall force. Med. Biol. Eng. Comput. **18**, 133—144

Izakov V. Y., Itkin G. P., Markhasin V. S. (1981): Biomechanics of cardiac muscle. Nauka, Moskow, p. 325 (in Russian)

Izakov V. Y., Katsnelson L. B., Blyakhman F. A., Markhasin V. S., Shklyar T. F. (1991): Cooperative effects due to calcium binding by troponin and their consequences for contraction and relaxation of cardiac muscle under various conditions of mechanical loading. Circ. Res. **69**, 1171—1184

Katsnelson L. B., Markhasin V. S. (1996): Mathematical modeling of relations between the kinetics of free intracellular calcium and mechanical function of myocardium. J. Mol. Cell. Cardiol. **28**, 475—486

Katz A. M. (1988): Molecular biology on cardiology: a paradigmatic Shift. J. Mol. Cell. Cardiol. **20**, 355—366

Katz A. M., Katz P. B. (1989): Homogeneity out heterogeneity. Circulation **79**, 712—717

Klausner S. C., Blair T. J., Bulawa W. F., Jensen G. M., Jeppson R. L., Clayton P. D. (1982): Quantitative analysis of segmental wall motion through systole and diastole in the normal human left ventricle. Circulation **65**, 580—590

Landesberg A., Sideman S. (1994): Coupling calcium binding to troponin C and cross-bridge cycling in skinned cardiac cell. Am. J. Physiol. **266**, H1260—1271

Landesberg A., Markhasin V. S., Beyar R., Sideman S. (1996): Effect of cellular inhomogeneity on cardiac tissue mechanics based on intracellular control mechanisms. Am. J. Physiol. **270**, H1101—1114

Lew W. Y., Rasmussen C. M. (1989): Influence of nonuniformity on rate of left ventricular pressure fall in the dog. Am. J. Physiol. **256**, H222—232

LeWinter M. M., Kent R. S., Kroener J. M., Carew T. E., Covell J. W. (1975): Regional differences in myocardial performance in the left ventricle of the dog. Circ. Res. **37**, 191—199

Markhasin V. S., Glasman A. A., Chestukhin V. V., Katsnelson L. B., Goldberg S. I., Makhanek A. O. (1994): The contribution of the segmentary nonhomogeneity of the myocardium of the left ventricle walls into its contractile and pumping function. Fiziol. Zh. **18**, 72—79 (in Russian)

Markhasin V. S., Katsnelson L. B., Nikitina L. V., Protsenko Y. L. (1997a): Mathematical modelling of the contribution of mechanical inhomogeneity in the myocardium to contractile function. Gen. Physiol. Biophys. **16**, 101—137

Markhasin V. S., Rutkevich S. M., Nikitina L. V., Protsenko Y. L. (1997b): A method for assessing the mechanical characteristics of the contractile element in isolated myocardial preparations. Ross. Fiziol. Zh. im I. M. Sechenova **83**, 136—139 (in Russian)

Noble E. G., Dabrowski B. L., Jamuzzo C. D. (1983): Myosin transformation in hypertrophied rat muscle. Pflügers Arch. **396**, 260—262

Pandian N. G., Skorton D. J., Collins S. M. (1983): Heterogeneity of left ventricular wall thickening and excursion in 2-dimensional echocardiograms of normal human subjects. Am. J. Cardiol. **51**, 1667—1675

Panerai R. B. (1980): A model of cardiac muscle mechanics and energetics. J. Biomech. **13**, 929—940

Peterson J. N., Hunter W. C., Berman M. R. (1991): Estimated time course of Ca^{2+} bound to troponin C during relaxation in isolated cardiac muscle. Am. J. Physiol. **260**, H1013—1024

Samuel J. L., Rappaport L., Mercadier J. J. (1983): Distribution of myosin isoenzymes within single cardiac cells. Circ. Res. **52**, 200—209

Sheehan F. H., Stewart D. K., Dodge H. T., Mitten S., Bolson E. L., Brown B. G. (1983): Variability in the measurement of regional left ventricular wall motion from contrast angiograms. Circulation **68**, 550—559

Schafer S., Friedler V. B., Thamer V. (1992): Afterload dependent prolongation of left ventricular relaxation: importance of asynchrony. Cardiovasc. Res. **26**, 631—637

Tennant L. R., Wiggers C. J. (1935): The effect of coronary occlusion on myocardial contraction. Am. J. Physiol. **112**, H351—361

Tyberg J., Parmley W. W., Sonnenblick E. H. (1969): In-vitro studies of myocardial asynchrony and regional hypoxia. Circ. Res. **25**, 569—579

Whallen R. (1985): Myosin isoenzymes as molecular markers for muscle physiology. J. Exp. Biol. **115**, 43—53

Wiegner A. W., Allen G. J., Bing O. H. (1978): Weak and strong myocardium in series: implications for segmental disfunction. Am. J. Physiol. **235**, H776—783

Final version accepted: April 23, 20002