NEW BIOMEDICAL TECHNOLOGIES

Role of Actin and Myosin in the Mechanism of the Decrease of Myocardial Contractility and Efficiency of Energy Transformation by Myocardial Myofibrils in Chronic Heart Failure in Humans

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Experiments with hybrid myocardial fibers showed that abnormalities of actin (basic protein of fine sarcomer threads) are responsible for reduced contraction rate, decreased developed force, and low efficiency of cardiomyocyte contraction in chronic heart failure caused by dilatation and ischemic cardiomyopathies and infective allergic myocarditis. Wastefulness of the contractile process in cardiomyocyte under conditions of pronounced energy deficit play a key role in progression of chronic heart failure. Hence, actin hypothesis of reduced contractile activity of myocardial contractile protein system in acute heart failure transforms into the actomyosin concept in chronic heart failure.

Key Words: *chronic heart failure; hybrid myocardial fibers; energy transformation; force generation*

The idea of developing and introducing the method of cross hybridization of myofibril proteins in studies of the mechanism of myocardial contractility decrease appeared in 1971 because there was no method which could help elucidate the role of the basic contractile and minor proteins in force generation. It was demonstrated that replacement of myocardial actin in reconstructed actomyosin complex from a healthy individual with actin from a patient with chronic heart failure (CHF) led to a decrease in the rate and extent of actomyosin superprecipitation [2,3]. Similar to contraction, superprecipitation is a result of sliding of fine threads along thick ones [3,12,13]. The next step in evaluation of the role of actin and myosin in the generation of contraction force was hybrid myocardial fibers (HMF). These hybrid complexes in contrast to actomyosin macromolecules formed by the cross method retain natural structure and spatial orientation of the thin and thick threads. This allowed studies of all the main parameters of the contractile, enzymatic, and relaxation capacity of myofibrils [5,7]. We evaluated the specific contribution of basic proteins of fine and thick threads to the mechanism of reduction of force generated in the course of isometric contractions and to efficiency of energy transformation in patients with CHF by investigating the contractile and enzymatic characteristics of HMF.

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MATERIALS AND METHODS

The study was carried on myocardium specimens from 10 patients with CHF (mean age 62.1±2.8 years, mean

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functional class 3.2±0.3) caused by dilatation cardiomyopathy (n=5) and postinfarction cardiosclerosis combined with essential hypertension (n=5) and 7 normal subjects (mean age 54.2±2.6 years) died from acute injury incompatible with life at the site of accident.

Methods for preparing skinned myocardial fibers, HMF, shadow myocardial fibers (SMF; skinned myocardial fibers after myosin removal), and myosin, for measuring the velocity and extent of force generated by skinned myocardial fibers and HMF in the isometric mode at ionic strength 0.13, 25°C, pH 7.4, pCa 6.0 (contraction) and pCa>8 (relaxation) and at temperatures 5-37°C, pH 5.0-8.9, and pCa 8.0-4.0, evaluation of Ca,Mg-ATPase activity of myofibrils and myosin, estimation of changes in free energy ΔG of ATP hydrolysis, and methods of statistical processing of the results were described previously [5,7,11].

RESULTS

In normal human skinned myocardial fibers the generated tension and ATP hydrolysis are minimum at low pH and low temperature (the processes are inhibited), and maximum (activated) at high (physiological) temperature and alkaline pH. The transition from minimum to maximum is described by smooth S-shaped curves (Fig. 1, *a, d*).

In CHF, the tension developed by skinned myocardial fibers at low pH (5.0-5.4) and 5-10°C did not differ from the control. Instead of S-shaped increase of tension from minimum to maximum at increased pH and temperature the curves are characterized by low maximums at 20°C and pH 6.4-6.7 and subsequent smooth slope at pH 8.2-8.9 (Fig. 1, *a*) and 30-37°C (Fig. 1, *d*).

ΔG of ATP hydrolysis in CHF at 37°C is 4-fold below the control. The actomyosin ensemble hydrolyzed far less amounts of ATP in the entire range of electric charge changes (Fig. 1, *e*) irrespective of generated force. CHF is associated with complete functional dissociation of force generation centers and ATP hydrolysis: conjugation of r processes drops from 0.8 (p<0.001) for skinned myocardial fibers of normal human heart to 0.25 in CHF. This determined low efficiency of energy transformation: the P/ΔG ratio decreases from 2.25 (normal human myocardial myofibrils) to 0.8. The cardiomyocyte engine runs idle and system energy is primarily transformed into heat [3, 11]. Hence, CHF under conditions of high pH and normal temperature is associated with pronounced dysfunction of the actomyosin ensemble, which can less pronounced under experimental conditions (22-25°C).

In CHF, Ca sensitivity increases (Fig. 2, *a*) and cooperative Ca response drops (normally to 1.5, Fig. 1, *c*) without notable changes in the protein spectrum of myofibrils in CHF patient.

These results indicate that CNS leads to development of a deficiency in the power generating complex, as a result of which the force does not increase with increase of pH and temperature (as it normally occurs), but decreases even in normal values of these parameters. ATP hydrolysis ΔG is no longer regulated and the force generation process becomes wasteful. This conclusion is practically important: it dictates urgent decrease of temperature if it is increased in CHF patients and suggests that cardioplegia exerts a cardioprotective effect not only due to deceleration of the metabolic processes, but also due to prevention of the negative effects of high or even normal temperature on the structural conformation status of actin, actomyosin ensemble, promoting the preservation of the contractile reserve.

HMF reconstructed from SMF of patients with CHF and normal human myocardial myosin generated strain and hydrolyzed ATP with less intensity under conditions of isometric contractions than HMF formed from normal human SMF and myocardial myosin (Table 1), the efficiency of contractions being decreased 1.4 times, Ca sensitivity increased, and the cooperative Ca response decreasing similarly as with skinned myocardial fibers (Fig. 2, *b*), which was determined by changed properties of the cardiomyocyte sarcomer fine thread.

The next problem in elucidation of the role of actin in attenuation of myofibril contractile properties was to exclude the contribution of the tropomyosin-troponin complex. To this end, regulatory proteins were removed from SMF (up to 80-90%, according to PAAG-sodium dodecylsulfate electrophoresis, without changes in the proportion between light and heavy myosin chains). The pattern of contractile activity of HMF (reconstructed from SMF of a CHF patient) free of the tropomyosin-troponin complex (HMF^{-}) did not differ from that of native HMF (Table 1) except reduced contractile activity and ATP hydrolysis ΔG.

Tropomyosin function in the fine thread is believed to consist in stabilization of the structure and function of the actin subunits, enhancement of potent interaction of actin with the myosin head [5,15], integration of force generated by individual actomyosin ensembles along the fine thread, and transfer of integrated pooling effort to Z membrane [15]. The fact that rigor force generated by skinned myocardial fibers (Fig. 1, *c, f*) in health and CHF is virtually equal to active force (Fig. 1) supports this conclusion. Hence, impairment of the actin protomer properties underlies the decreased cooperation of the contractile process, intensity and velocity of force generation, and efficiency of energy transformation by the contractile protein system in CHF.

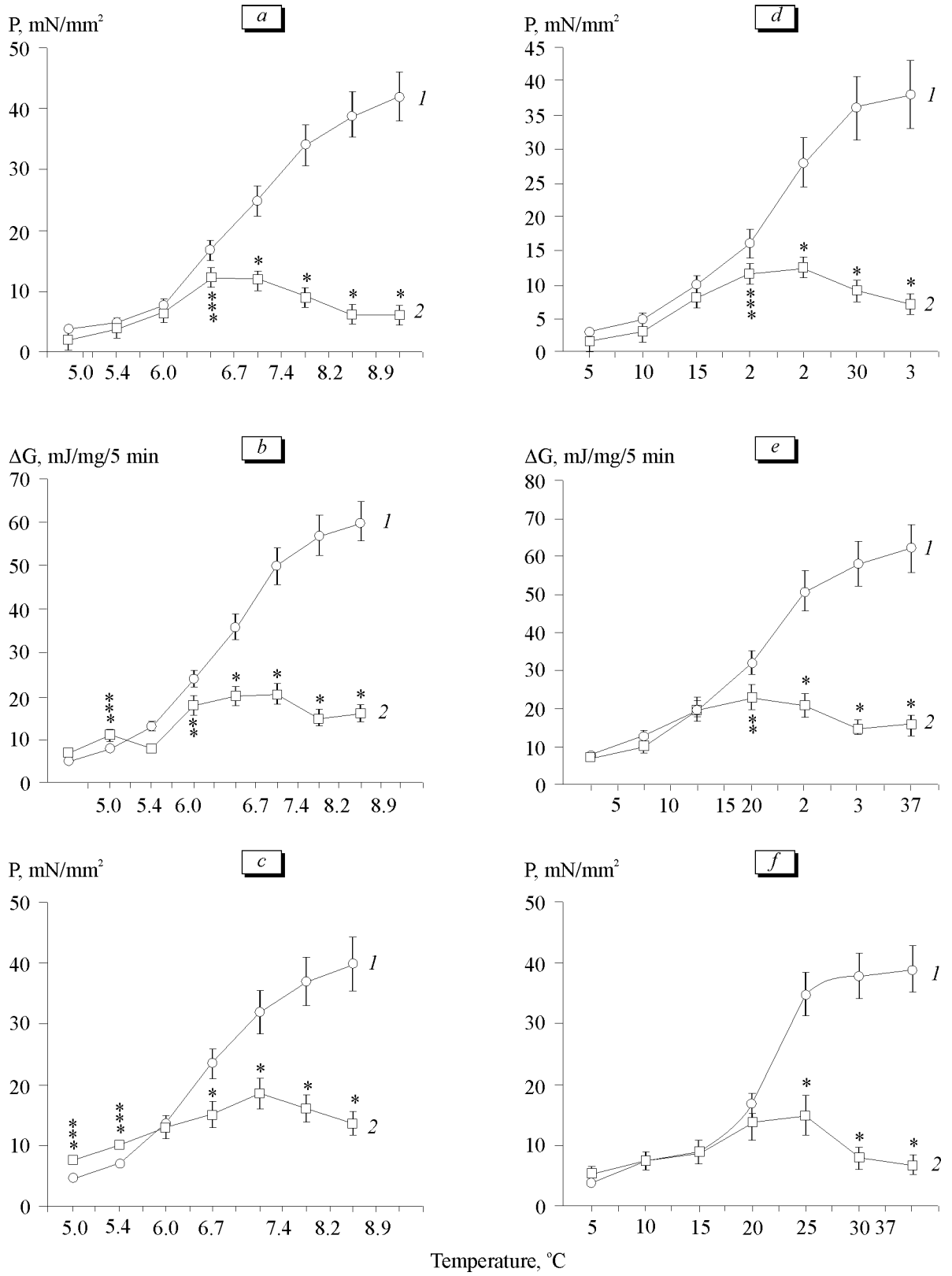

Fig. 1. Relationship between tension developed by skinned myocardial fibers during isometric contraction (*a, d*), ATP hydrolysis energy (*b, e*), and rigor force (*c, f*), on the one hand, and pH (*a-c*) and temperature, on the other (*d-f*). *1*) healthy humans; *2*) patient with chronic heart failure. Here and in Figs. 2 and 3: **p*<0.001, ***p*<0.01, ****p*<0.05 *vs.* normal human myocardial fibers.

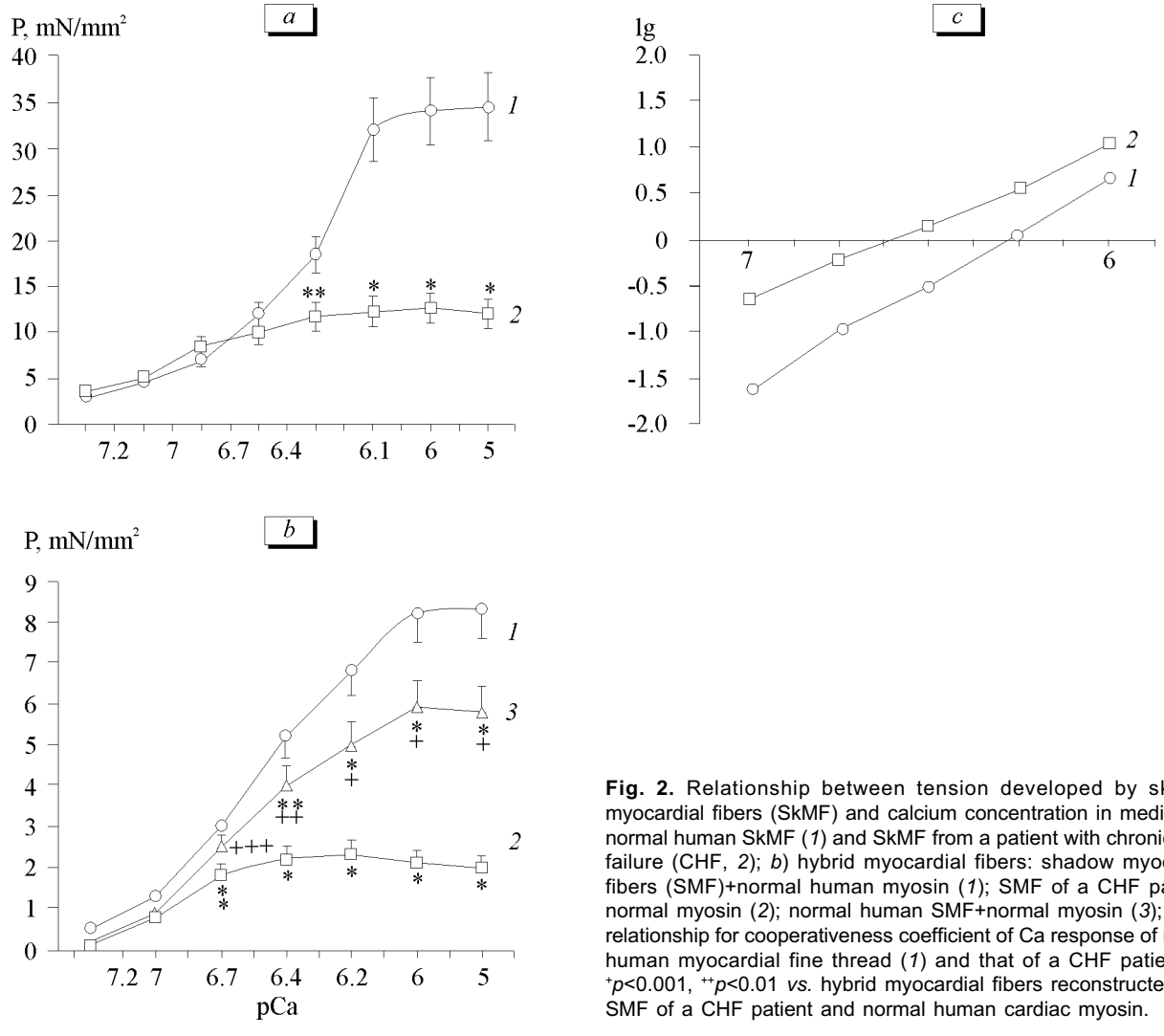

Fig. 2. Relationship between tension developed by skinned myocardial fibers (SkMF) and calcium concentration in medium. *a*) normal human SkMF (*1*) and SkMF from a patient with chronic heart failure (CHF, *2*); *b*) hybrid myocardial fibers: shadow myocardial fibers (SMF)+normal human myosin (*1*); SMF of a CHF patient+ normal myosin (*2*); normal human SMF+normal myosin (*3*); *c*) Hill relationship for cooperativeness coefficient of Ca response of normal human myocardial fine thread (*1*) and that of a CHF patient (*2*). $^{+}p<0.001$, $^{++}p<0.01$ *vs.* hybrid myocardial fibers reconstructed from SMF of a CHF patient and normal human cardiac myosin.

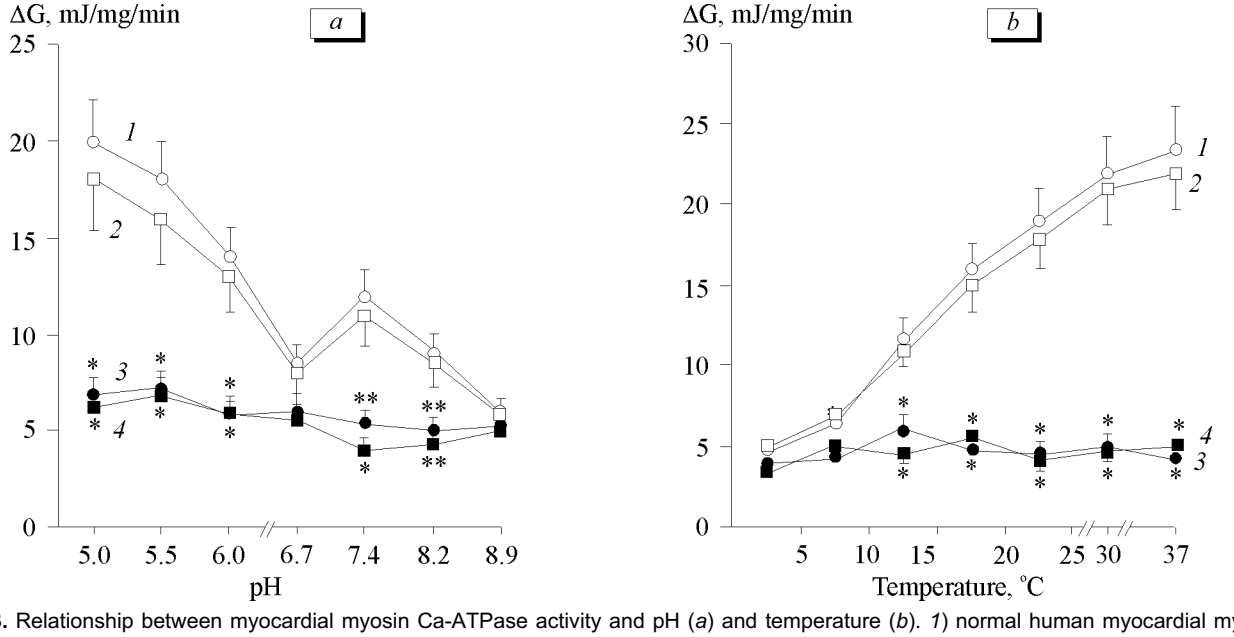

Fig. 3. Relationship between myocardial myosin Ca-ATPase activity and pH (*a*) and temperature (*b*). *1*) normal human myocardial myosin; *2*) normal rabbit myocardial myosin; *3*) CHF patient's myocardial myosin; *4*) myocardial myosin of a rabbit with toxic allergic myocarditis of 10-days duration.

TABLE 1. Tension, ATP Hydrolysis Energy, and Efficiency of Contractions of HMF Reconstructed from SMF/SMF^{-} and Myosin from a Patient with CHF (d) and Normal Subject (n)

Parameter	SMF-n+myosin-n	SMF-d+myosin-d	SMF-d+myosin-n	SMF-n+myosin-d
P, mN/mm^2	9.8	3.8*	3.95*	6.3**++
	6.2	2.2*	2.4*	4.5**+
ΔG, mJ/mg/5 min	28	16*	15*	20*++
	18	10*	10.5*	14.5*+
P/ΔG, mN/mD	0.82	0.48*	0.45*	0.65*+
	0.32	0.18**	0.16*	0.3**++

Note. *p<0.001, **p<0.01 compared to HMF from SMF-n+myosin-n; +p<0.001, ++p<0.01 compared to HMF from SMF-d+myosin-d.

In an alternative HMF formed from normal human heart SMF and myocardial myosin of a patient with CHF the size of developed tension and ATP hydrolysis ΔG decreased, similarly as with CHF patient's SMF and normal myosin (Table 1), but these changes were less pronounced and the efficiency of the contractile process decreased not by 40% but only by 14% (Fig. 2, *c*). The decrease of ATP hydrolysis ΔG and the generated tension did not correlate, therefore changes in the properties of skinned myocardial fibers in CHF were determined by changes in the properties of both actin and myosin. Alternative HMF formed from myocardial myosin of a CHF patient and normal human SMF retained Ca sensitivity, cooperative Ca response (Fig. 2, *b*), and, which is particularly important, the rate of relaxation, in contrast to the HMB containing SMF of a CHF patient. All this confirms that actin involvement plays the key role in the decrease of generated force, ATP hydrolysis ΔG, and efficiency of energy transformation by the system of myocardial contractile proteins [5,7,12,13].

Study of monomer actin confirmed that myosin properties changed in CHF: the curve of monomer myosin Ca-ATPase activity (in high ionic strength) was abnormal and stabilized at a low (baseline) level. In the acid pH range the myocardial myosin Ca-ATPase activity in CHF was lower than in the control (Fig. 3, *a*), while myocardial Ca-ATPase activity in health in high ionic strength and 25°C was characterized by a pronounced maximum at pH 5.0-6.0, drop of activity with the minimum at pH 6.7, second lower peak (1.5 times lower than the first peak) at pH 7.4, and a new drop with the minimum at pH 8.9 (Fig. 3, *a*).

Within a temperature range of 5-40°C the Ca-ATPase activity was described by an S-shaped curve (but not two-peak curve). In CHF and in heart failure caused by toxic allergic myocarditis the pattern of Ca-ATPase activity completely transforms with temperature rise: S-shaped pattern disappears and Ca-ATPase shows resistance to temperature in the entire 5-40°C interval (Fig. 3, *b*), *i.e.* myocardial myosin is no longer capable of modifying the conformation status and be adequately activated by actin.

Hence, the mechanism of CHF development is an intricate many-stage process of limitation of the contractile and relaxation capacity of the cardiomyocyte contractile system, development of a highly entropic, low energy, contracture state paralleled by decrease of the actomyosin ensemble capacity to release free energy of ATP hydrolysis which determines the extent of the maximum work performed by the system. However the mechanism of CHF development is not confined to disorders in the myocardial contractile proteins, which are paralleled by development of energy deficiency in the cells and disorders in Ca^{2+} transport system [3], this leading to the progress of destructive processes. These subcellular and molecular disorders develop in the presence and with involvement of ultrastructural disorders in all three systems of the cardiomyocyte, responsible for contraction-relaxation.

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