NEW BIOMEDICAL TECHNOLOGIES

Subcellular and Molecular Mechanisms of the Effects of Cardiac Glycosides and Angiotensin-Converting Enzyme Inhibitors on Contractile Function and Energy Conversion in Myocardial Myofibrils under Normal Conditions and during Acute Cardiac Insufficiency

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 1, pp. 87-93, January, 2002
Original article submitted January 12, 2001

Experiments on skinned and hybrid myocardial fibers isolated from normal dogs and animals subjected to 120-min occlusion of the anterior interventricular branch of the coronary artery showed that in contrast to cardiac glycosides, angiotensin-converting enzyme inhibitors suppress contractile ability of myocardial myofibrils in a dose-independent manner within the concentration range of 10^{-12}-10^{-4} M. This effect is accompanied by a decrease in fiber relaxation rate most pronounced in the presence of captopril. Actin, the major protein of fine filaments is the target for β-acetyldigoxin, K-strophanthin, captopril, enalapril, and trandolapril in myocardial myofibrils. During coronary occlusion, the inhibitors of angiotensin-converting enzyme induce structural and conformational changes in actin that decrease efficiency of contraction. The data obtained cast doubt on advisability of therapeutic use of angiotensin-converting enzyme inhibitors in the therapy of myocardial infarction, especially in its early period.

Key Words: *angiotensin-converting enzyme inhibitors; cardiac glycosides; cardiomyocyte myofibrils; energy conversion; force generation*

At present, angiotensin-converting enzyme inhibitors (ACEI) are considered as the first-line drugs in the treatment of acute and chronic cardiac insufficiency caused by acute myocardial infarction. However, at the tissue level (myocardial strips), ACEI exert not the positive inotropic effect typical of cardiac glycosides, non-glycoside inotropic preparations, angiotensin II, norepinephrine, and dobutamine, but the negative one [9,15]. However, the subcellular and molecular mechanisms of ACEI action on cardiomyocytes remain unclear. Our aim was to study the effects of cardiac glycosides and ACEI on contraction and relaxation of myocardial myofibrils at the molecular and submolecular levels under normal conditions and during acute cardiac insufficiency provoked by 120-min coronary artery occlusion (CAO).

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0007-4888/02/1331-0074$27.00

MATERIALS AND METHODS

The study was carried out on myocardial contractile protein system (MCPS), skinned myocardial fibers (SMF), hybrid myocardial fibers (HMF) formed from ghost myocardial fibers (GMF, myosin-free fibers composed of natural fine actin filaments, tropomyosin, and troponin), and GMF lacking troponin-tropomyosin complex (GMF^{-}) that preserve natural spatial orientation. The specimens were obtained from the hearts of normal dogs and from myocardial ischemic area in dogs subjected to occlusion of the upper one-third of the anterior interventricular branch of left coronary artery by routine methods [7,9].

The amplitude and rate of tension generation during isometric contraction, Ca,Mg-ATP activity of myosin in SMF and HMF, the changes in free energy of ATP hydrolysis (ΔG), and the content of free Ca^{2+} ions in the medium were determined as described previously [2,4,5]. To reveal the target of cardiac glycosides or ACEI in the myocardium, we studied the properties of SMF and HMF formed from myosin and GMF preincubated with cardiac glycosides or ACEI. Alternatively, the examined fibers were formed from untreated GMF and myosin preincubated with the drugs. In all experiments, after preincubation of myocardial fibers with the drug, they were washed with drug-free medium. Preincubation of GMF, GMF^{-}, myosin, and actin with cardiac glycosides or ACEI, and also the measurements of orientation and light emission by fluorescent probes to Cys374 (N-iodoacetyl-N'-(5-sulfo-1-naphthyl)ethylene amine, 1,5-I-AEDANS), Lys61 (fluorescein 5-isothiocyanate, FITC), and Cys10 and Tir69 (5(iodoacetamido)fluorescein) were performed as described elsewhere [2,4,6]. Actin polyme-

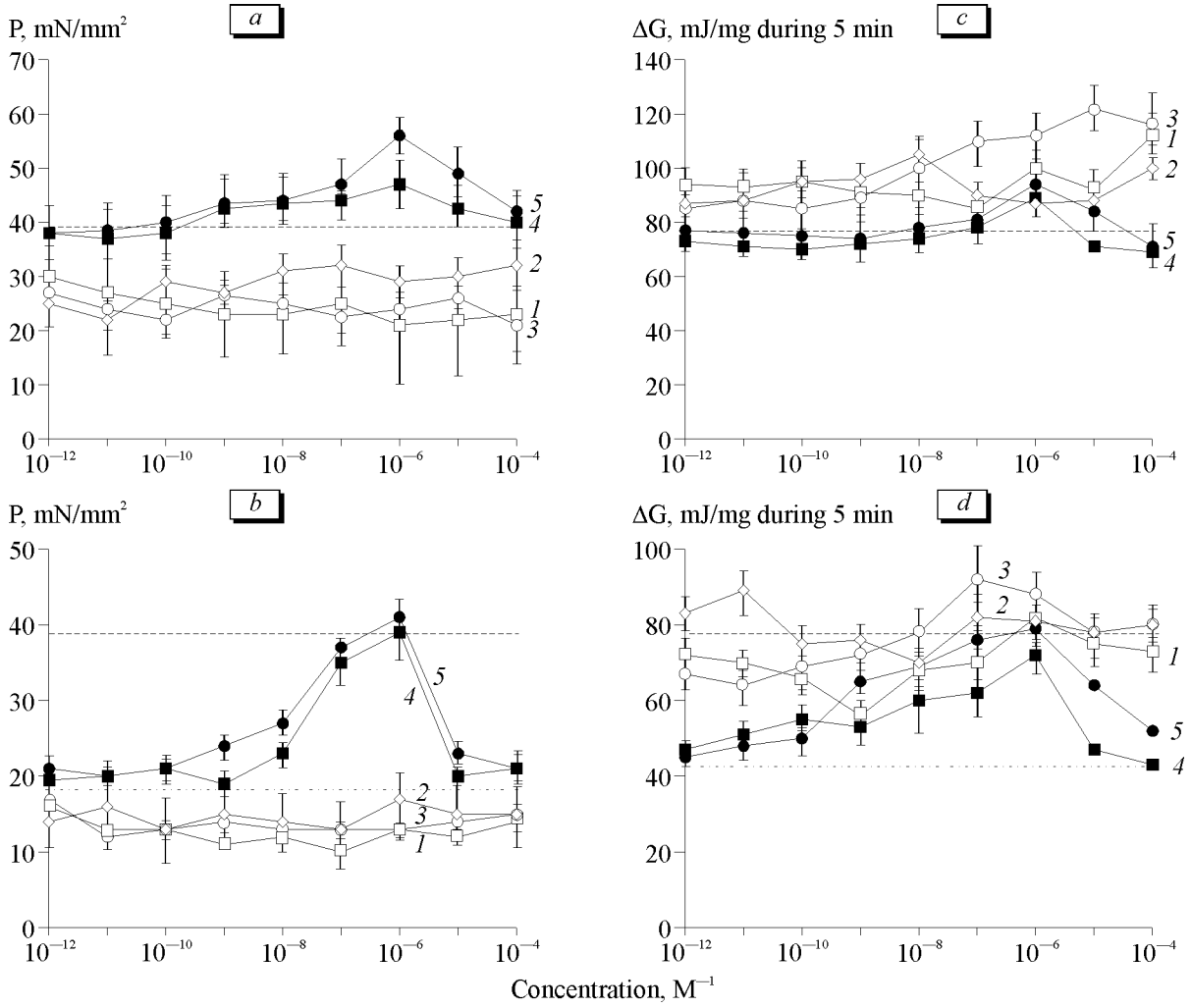

Fig. 1. Effect of enalapril (*1*), captopril (*2*), trandolapril (*3*), β-acetyldigoxin (*4*), and strophanthine K (*5*) on the amplitude of generated force (*a*, *b*) and energy of ATP hydrolysis (*c*, *d*) under normal conditions and during acute cardiac insufficiency caused by 120-min myocardial ischemia. The dashed and chain lines show the drug-free values measured under normal conditions and during ischemia, correspondingly.

TABLE 1. Tension, Energy of ATP Hydrolysis, and Efficiency of Contraction in HMF Formed from GMF (Numerator) and GMF^{-} (Denominator) of Normal (N) and Ischemic (I) Myocardium

Index	Drug-free	Myosin+ strophanthin K	Myosin+ captopril	GMF+ strophanthin K	GMF+ captopril
P, mN/mm^2					
GMF-N+myosin-N	9.9±0.4 / 6.6±0.4	10.0±0.5 / 6.4±0.2	9.7±0.4 / 6.7±0.3	13.0±1.5* / 7.5±0.3**	6.7±1.0*$^{+}$ / 4.6±0.4**$^{++}$
GMF-N+myosin-I	8.9±0.3 / 6.7±0.3	— / —	— / —	8.8±0.3 / 6.75±0.25	8.9±0.3 / 6.67±0.13
GMF-I+myosin-I	3.2±0.2 / 2.0±0.3	— / —	— / —	7±2* / 3.4±0.4**	2.25±0.15*$^{++}$ / 1.65±0.15*$^{+++}$
ΔG, mJ/mg during 5 min					
GMF-N+myosin-N	28±2 / 23±3	30±3 / 25±2	30.5±1.5 / 28±4	34.5±2.5*** / 31±3**	36±4** / 34±2*
GMF-N+myosin-I	31±4 / 21.5±1.5	— / —	— / —	28.7±2.7 / 20±2	30.5±2.5 / 19±2
GMF-I+myosin-I	18±3 / 17±3**	— / —	— / —	20.3±2.3* / 19±2*	16±2*$^{+++}$ / 21±2*$^{+++}$
Efficiency of contraction, mN/J					
GMF-N+myosin-N	0.8±0.1 / 0.35±0.05	0.78±0.13 / 0.33±0.04	0.77±0.12 / 0.35±0.03	0.95±0.10** / 0.40±0.05**	0.6±0.1**$^{++}$ / 0.24±0.04*$^{++}$
GMF-N+myosin-I	0.79±0.05 / 0.36±0.03	— / —	— / —	0.76±0.04 / 0.33±0.03	0.80±0.03 / 0.32±0.02
GMF-I+myosin-I	0.46±0.03 / 0.18±0.02	— / —	— / —	0.7±0.1* / 0.40±0.05**	0.27±0.03* / 0.15±0.03*$^{+++}$

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the norm; ^{+}p<0.001, ^{++}p<0.01, ^{+++}p<0.05 compared to strophanthine K.

rization was performed in the presence of strophanthin K and β-acetyldigoxin (Boehringer Mannheim) or captopril (Merck& Sharp&Dohme), enalapril maleate (Chemo Iberica), and trandolapril (Knoll AG).

The data were processed statistically using Student's t test.

RESULTS

Under normal conditions and during *in vitro* isometric contraction, β-acetyldigoxin and strophanthin K produced a dose-dependent increase in the force generated by SMF of intact heart (Fig. 1, *a*). The maximum effect was observed at a concentration of 10^{-6} M. Further increase of concentration decreased the force to the initial value, this effect being most pronounced in experiments with β-acetyldigoxin. The same regularity was observed in respect to ΔG of ATP hydrolysis (Fig. 1, c). Strophanthin K 1.3-fold increased the contraction efficiency (the highest value), while β-acetyldigoxin was ineffective.

In CAO β-acetyldigoxin and strophanthin K applied in concentrations of 10^{-9}-10^{-4} M dose-dependently increased the generated force and ΔG of ATP hydrolysis (Fig. 1, *b*). Thus, the force generated by SMF isolated from ischemic zone, ΔG of ATP hydrolysis, and efficiency of contraction returned to normal. Further increase in concentration of cardiac glycosides decreased all indices of SMF contraction to the level observed in drug-free experiments with CAO. Therefore, cardiac glycosides normalize contraction (Fig. 1, *c*) and relaxation of MCPS in the ischemic zone *in vitro*.

When ACEI captopril (n=12), enalapril (n=6), and trandolapril (n=5) were applied to normal myofibrils in the concentration range of 10^{-12} to 10^{-4} M, their contractile activity pronouncedly decreased (Fig. 1, *a*). The ACEI-induced decrease of the developed tension was accompanied not by a proportionate decrease in ΔG of ATP hydrolysis (Fig. 1, c), but by a pronounced activation of Ca-Mg-ATPase in myofibrils, which was maximum at ACEI concentrations of 10^{-6}-10^{-4} M (*per os* administration). As a result, in contrast to the effect of cardiac glycosides, efficiency of force generation by SMF from normal heart was not enhanced by the examined ACEI, but markedly decreased (by 35%), the effect being most pronounced for trandolapril. At

the same time, sensitivity to Ca^{2+} and cooperativity of contractile process in SMF did not change, while maximum rate of SMF relaxation decreased 1.30-, 1.25-, and 1.20-fold under the effect of captopril, enalapril, and trandolapril, correspondingly.

In ischemized heart, ACEI drugs (10^{-12}-10^{-4} M) produced a further decrease in contractile ability of cardiomyocyte myofibrils from ischemic area, and this effect did not depend on the dose (Fig. 1, *b*). Similar to its effect in intact fibers, ACEI markedly decreased the maximum relaxation rate of ischemic fibers after isometric contraction, the effect of captopril being most pronounced (1.4-fold). Drastic decrease in the rate and value of the force generated by MCPS induced by ACEI under normal conditions or during CAO was not accompanied by a proportional decrease in ΔG of ATP hydrolysis. By contrast, it was accompanied by pronounced activation of Ca-Mg-ATPase relatively to the level measured during CAO. As a result, the contractile process in SMF, which is inefficient during acute cardiac insufficiency, became even more wasteful under the action of examined ACEI drugs.

HMF reconstructed of GMF from ischemic heart of the dog subjected to CAO and myosin from normal myocardium generated pronouncedly smaller force than HMF composed of GMF from normal heart and myosin isolated from CAO-produced ischemic area or normal myosin (Table 1). Moreover, attenuation of generated force in such HMF, as in SMF from ischemic area, was accompanied by a pronounced decrease in contraction efficiency. Complete uncoupling of force generation and ATP hydrolysis (r=0.16) was observed in hybrid actomyosin ensembles containing fine filaments isolated from ischemic area of the heart subjected to CAO and normal myosin, while these process were closely coupled in HMF from normal heart and myosin isolated from CAO-ischemized or intact myocardium as in the case of SMF from normal heart (r= 0.83 and r=0.85, correspondingly; p<0.001).

HMF reconstructed of GMF^{-} isolated from CAO-ischemized myocardium and normal myosin and HMF formed from GMF containing regulatory proteins generate 4.2-fold and 2.8-fold weaker force with correspondingly 3.5-fold and 2.4-fold slower rate. ATP hydrolysis and force generation in HMF containing GMF^{-} isolated from ischemic area or natural GMF^{-} were also completely uncoupled (r= 0.23 and r=0.41, correspondingly), which attests to deep disturbance of energy conversion in actomyosin ensemble resulted from changes in the properties of basic protein of fine actin filament and alteration of its conformation state [3].

When normal myosin was incorporated into GMF or GMF^{-}, which were isolated from CAO-produced ischemic area and preincubated with cardiac glycosides, generated force and ΔG of ATP hydrolysis increased resulting in elevation of contraction efficiency (Table 1). These data indicate that the target of cardiac glycosides during acute myocardial infarction is actin, the basic protein of fine filament in sarcomeres of myocardial myofibrils.

HMF formed from GMF of normal heart treated with examined ACEI drugs and myosin isolated from normal myocardium (not treated with ACEI) generated smaller isometric tension than the control

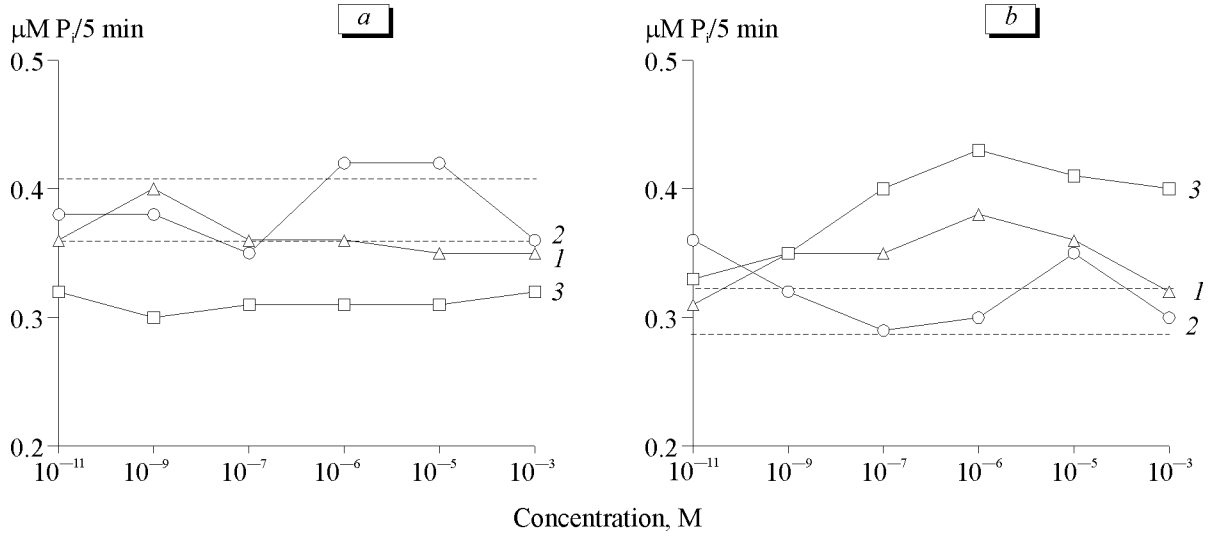

Fig. 2. Effect of β-acetyldigoxin (*1*), capoten (*2*), and trandolapril (*3*), on myosin Ca-ATPase in normal (*a*) and ischemic myocardium subjected to a 120-min acute ischemia (*b*). The dashed lines limit the region of index value measured in drug-free conditions. P_i is inorganic phosphate.

TABLE 2. Effect of β-Acetyldigoxin and Enalapril on the Changes of Distances (nm) between Different Amino Acid Residues in Monomer (n=6) and Protomer (n=5) of Actin Filament ($M \pm m$)

Amino acid residues	Control	Experimental conditions	
		β-Acetyldigoxin	Enalapril
Cys374-Lys61			
Monomer			
norm	4.70±0.10	4.31±0.08***	4.92±0.12***^{xx}
CAO	5.32±0.08*	4.89±0.11^{oo}	5.80±0.13**^{ooxx}
Protomer			
norm	3.90±0.09^{+}	4.12±0.08***	4.75±0.11**^{xx}
CAO	5.32±0.08*	4.19±0.11^{o}	5.80±0.13*^{ox}
Adjacent protomers			
norm	3.80±0.10	3.90±0.10	4.12±0.12***
CAO	4.15±0.10**	4.05±0.11	4.16±0.13***
Cys374-Tir69			
Monomer			
norm	2.65±0.11	2.44±0.10	2.87±0.10***
CAO	3.25±0.12*	2.67±0.11^{oo}	3.33±0.13**^{xx}
Protomer			
norm	1.96±0.12^{+}	1.72±0.08***	1.90±0.11
CAO	2.65±0.10*	2.20±0.12^{oo}	2.85±0.15**^{oo}
Adjacent protomers			
norm	4.10±0.09	3.90±0.10	4.12±0.12
CAO	4.35±0.06	3.85±0.11*	4.46±0.13***
Cys374-Cys374			
Adjacent protomers			
norm	4.68±0.08	4.46±0.09*	4.82±0.10
CAO	5.26±0.11*	4.85±0.11^{oo}	5.46±0.12*^{xx}

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the control; ^{+}p<0.001 compared to monomer; ^{o}p<0.001, ^{oo}p<0.01 compared to the norm; ^{x}p<0.001, ^{xx}p<0.01 compared to β-acetyldigoxin.

HMF — by 40, 29, and 33%, correspondingly (Table 1). Instead of beneficial decrease in ΔG of ATP hydrolysis, ACEI increased it, which means a significant drop in contraction efficiency (Table 1). When HMF was formed from ischemic GMF preincubated with ACEI and normal myosin not treated with ACEI, the value of generated force also decreased, which resulted in additional drop in contraction efficiency (Table 1). Attenuation of generated force and increase of ΔG of ATP hydrolysis in ACEI-treated HMF was also observed in experiments with GMF^{-} (Table 1). Therefore, attenuation of generated force and increase of ΔG of ATP hydrolysis induced by ACEI were caused by direct action of these drugs on actin.

Ca- and K-EDTA-ATPase activity of purified monomeric myosin did not change in the entire range of examined ACEI concentrations (Fig. 2). The change of myosin in HMF formed from ischemic GMF and normal myosin by ischemic myosin preincubated with cardiac glycosides or ACEI produced no significant effect on generated force, ΔG of ATP hydrolysis, and contraction efficiency. Therefore, the increase in intensity of ATP hydrolysis by MCPS preincubated with cardiac glycoside or ACEI was caused not by the changes in the myosin ATPase properties (Fig. 2), but by potentiation of actin-induced activation of myosin in actomyosin and enhancement of Ca,Mg-ATPase activity of actomyosin resulting from the changes of actin properties, because cardiac glycosides and ACEI produced no changes in myosin properties.

During CAO-produced acute cardiac insufficiency, the changes in fluorescence intensity of 1,5-I-AEDANS-FITC-labeled actin induced by β-acetyldigoxin (n=6) or strophanthine K (n=5) were similar to those produced by cardiac glycosides on myocardial actin during early cardiac insufficiency

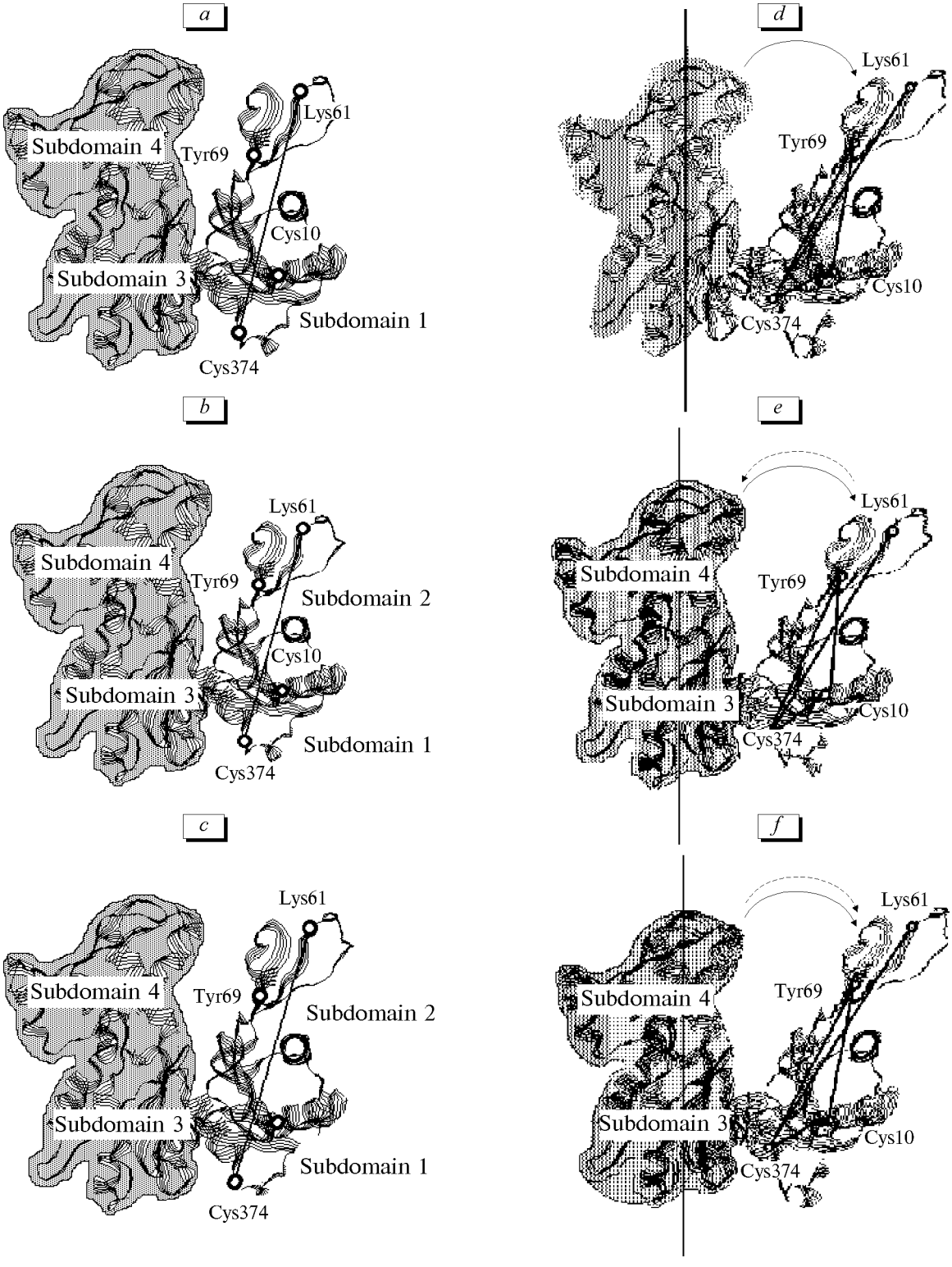

Fig. 3. Hypothetical model of actin monomer under normal conditions (*a-c*) and in acute cardiac insufficiency (*d-f*). *a, d*) without drugs; *b, e*) cardiac glycosides; *c, f*) inhibitors of angiotensin-converting enzyme. Solid arrows show directivity of the changes during acute cardiac insufficiency, dash arrows mark the changes under the effect of the drug.

[7]. Cardiac glycosides shortened the distance between Cys374 and Lys61 in ischemic area to normal value both in actin monomer and protomer of actin filament (Table 2). In addition, changes in the area occupied by Tir69 became more pronounced (Table 2, Fig. 3). These changes and normalization

of radial distance to Cys374 and the distance between Tir69 and Cys374 in the adjacent protomers of actin filament suggest that cardiac glycosides change the actin filament in ischemic area thereby promoting normalization of its conformation. By contrast, ACEI increased the distance between Cys374 and Lys61 both in monomer (n=6) and protomer (n=5) of actin filament, as well as the distance between Cys374 and Lys61 in the adjacent protomers to even greater extent than acute cardiac insufficiency (Table 2).

Thus, actin is the target for cardiac glycosides and ACEI. The structural and conformational changes in actin produced by cardiac glycosides and ACEI affect the center of interaction between actin and myosin thereby increasing or decreasing contraction efficiency, correspondingly. In other words, the pathological changes developed in myocardial myofibrils during acute cardiac insufficiency are aggravated by ACEI.

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