EFFECT OF VERAPAMIL ON MOLECULAR CHARACTERISTICS OF MYOCARDIAL ACTOMYOSIN IN EXPERIMENTAL ISCHEMIA

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Hypertrophy of the myocardium in various forms of oveloading of the heart can be prevented by means of β-blockers and Ca-antagonists [6, 8]. However, it is not yet clear whether the prevention of hypertrophy is connected with intensification of processes affecting the pool of metabolic reserves of the myocardium, or with protein transformation of the contractile structures, increasing the power of the muscle fibers. Considering the important role of changes in the intracellular Ca^{++} concentration in the regulation of metabolism of the myocardial cell and protein synthesis [1, 10], and on the basis of data showing the great importance of an increase in transmembrane entry of Ca^{++} inside the myocardial cell, it can be postulated that Ca^{++}-antagonists possess a broader spectrum of action in relation to the intracellular mechanisms of the adaptive function of the myocardium than has hitherto been supposed.

To elucidate the ways whereby verapamil exerts its prophylactic action, an attempt was made to detemine, on the one hand, the physicochemical properties of the actomyosin complex and, on the other hand, the probability of a change in quantitative synthesis of individual components of this macromolecule in the mechanism of action of the Ca-antagonist.

EXPERIMENTAL METHOD

Myocardial ischemia was induced by ligation of the left descending branch of the coronary artery in 60 male albino rats weighing 170-180 g. The animals were divided into three groups with 20 rats in each group: group 1) intact rats (control), group 2) animals with experimental myocardial ischemia (EMI); group 3) rats receiving verapamil in a dose of 200 μg/kg daily for 4 days after ligation of the coronary artery. The animals were kept under identical conditions in the animal house and decapitated on the 5th day after creation of EMI under ether anesthesia. Actomyosin (AM) was obtained from the nonischemic part of the left ventricle by the method in [13]. Mg^{++}-ATPase activity was determined by the method in [11]. Electrophoresis of protein was carried out in 10% polyacrylamide gel with sodium dodecylsulfate by the method in [15]. Dependence of the Mg^{++}-ATPase activity of AM on the Ca^{++} concentration in the medium was determined by the use of 1 mM Ca^{++}/EGTA buffer at pCa = 7-4. The degree of cooperativeness of the ATPase reaction was calculated by Hill's equation: $\log V = \text{const} + h \log [S]$, where h denotes the tangent of the angle of inclination between coordinates of $\log \left[\frac{V}{(V - v)}\right]$ and $\log [S]$.

EXPERIMENTAL RESULTS

On the 5th day of EMI basal ATPase activity of AM did not differ from the control values (Table 1). In the presence of Ca^{++} ions in sufficient concentration to saturate low-affinity sites on the macromolecule ($pCa^{++} \leqslant 4$), Mg^{++}-ATPase activity was depressed by 75%. Meanwhile, during treatment with verapamil, the ATPase activity of AM was considerably higher than in the control. Treatment of EMI with verapamil thus not only prevented a decrease in ATPase activity, but also increased its basal activity. It can accordingly be postulated that verapamil prevents changes in the contractile proteins themselves.

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Group of animals	In absence of Ca^{++}	P	In presence of Ca^{++}	P
1-я	1,72±0,0854	—	3,2±0,05	—
2-я	1,36±0,134	>0,05	0,77±0,071	<0,001
3-я	2,085±0,075	<0,001	5,35±0,118	<0,001

Legend. Mean results of 10 experiments.

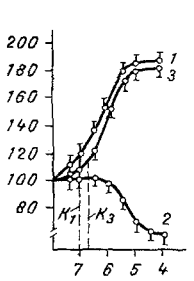

Fig. 1

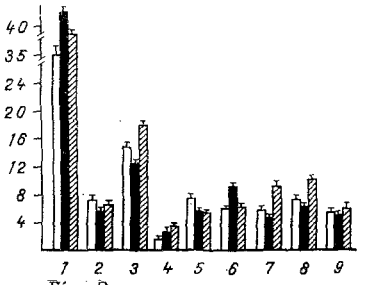

Fig. 2

Fig. 1. Ca^{++}-dependence of Mg^{++}-ATPase of AM. Abscissa, pCa (7-4); ordinate, ATPase activity (in %). 1) Control; 2) EMI; 3) EMI plus treatment with verapamil. Incubation medium contained: 0.5 mg/ml of AM, 1 mM Ca^{++}/EGTA buffer; 0.15M KCl; 0.2 mM Tris-HCl; 0.2 mM Mg^{++}-ATP; 4 mM dithiothreitol, pH 7.35 (25°C).

Fig. 2. Changes in composition of components of AM under the influence of verapamil. Abscissa: 1) myosin heavy chains, 2) α-actinin, 3) actin, 4) troponin T, 5) tropomyosin, 6) troponin I, 7) MLC (Myosin light chain)-1, 8) MLC-2, 9) troponin C; ordinate, concentration of components (in %). Unshaded columns — control, black columns — EMI, obliquely shaded columns — EMI + verapamil.

More complete information on possible changes in the protein macromolecule was given by the results of measurement of the Ca-reactivity of AM. In animals of the control group, with Ca^{++} concentrations initiating interaction of action with myosin, an almost twofold increase in ATPase activity was observed (Fig. 1). Meanwhile, in EMI the course of the Ca-dependence curve of the enzyme was considerably disturbed. Inhibition of Mg^{++}-ATPase was observed limits of pCa of 6.5-4. The cooperativeness of the process, expressed as the characterisitc shape of the curve recorded for AM preparations from intact animals, was completely lost. Restoration of the course of the Ca-dependence curve of the ATPase reaction during treatment with verapamil is an interesting fact. In this case almost complete restoration of the course of the curve was observed. The half-maximal activation of the enzyme K-Ca_{50}% was pCa = 6.6, which is close to the value of K_1 for AM preparations from control animals, for which pCa = 7.0. These facts indicate enlargement of the Ca-binding sites on the protein macromolecule, for the degree of cooperativeness of the ATPase reaction also was significantly increased: h = 1.7 compared with the control, for which h = 1.5.

The results of electrophoretic investigation of the composition of the protein components of AM showed that on the 5th day of EMI there was a significant increase in the relative content of myosin heavy chains and troponin T (TrT) (Fig. 2). The content of actin, tropomyosin, myosin light chains (MLC-1 and MLC-2), and of troponin C was reduced. The relative content of troponin I, an ATPase inhibitor, was increased. Incidentally, the increase in the relative content of troponin I agreed well with data showing a decrease in Mg^{++}-ATPase activity, for

the very small fluctuations in the composition of components of the macromolecule, namely troponin C, MLC, and MHC, could not evidently give rise to significant changes in enzyme activity of AM.

After treatment with verapamil, the relative content of troponin I was restored to its initial level, and this was accompanied by an increase in ATPase activity (Fig. 2; Table 1).

However, under the influence of verapamil the relative content of the other components of AM was changed and, in particular, there as an increase in the content of MLC-1, MLC-2, and troponin C compared with the control, in agreement with data showing positive correlation between the Ca^{++} level and ATPase activity and MLC-2 content during investigation of positively and negatively inotropic agents in the perfused rat heart [7]. The therapeutic action of verapamil in this respect is perhaps manifested as activation of Ca^{++}-calmodulin-dependent MLC-2 kinase [14]; it increases Ca^{++} transport through the sarcolemma, through slow Ca-channels at the time of depolarization [5]. Restoration of ATPase activity evidently reflects increased ability of the trans-sarcolemmal Ca^{++} to penetrate into the cardiomyocytes.

Similarity between membrane channels and adrenoreceptors has been discovered in recent years [2, 7]. It was shown previously [12] that the influence of adrenergic compounds on the troponin-tropomyosin complex, with respect to their activating action on ATPase of AM is similar to the influence of Ca^{++} ions, i.e., adrenergic agonists and antagonists exert their action at the molecular level through modulation of the functions of the Ca-binding sites of regulatory components (troponin C, calmodulin, MLC-2) of different oligomeric enzymes. It has also been shown that Ca-antagonists of the nifedipine type can interact with Ca-binding proteins, such as calmodulin [3] and, consequently, troponin C and MLC-2.

The mechanism of the therapeutic action of verapamil on ATPase of AM in ischemia can be explained on the basis of recently published data showing that certain Ca-antagonists, such as cinnarizine and trifluoperazine, which have lipophilic properties, on penetrating into myocytes can affect Ca^{++}-activation of the contractile proteins [3. 4]. Consequently, our results can be explained also by the direct action of verapamil on ATPase of AM through binding with Ca-sensitive sites on the contractile protein macromolecule. However, the possibility of its effect on metabolism, and also on expression of genetic loci responsible for synthesis of individual polypeptide chains cannot be ruled out.

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