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Rhythminotropic Reactions of Human Myocardium in Ischemic and Rheumatic Heart Diseases against the Background of Amiodarone

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Abstract

In human heart failure, Ca^{2+} homeostasis gets disturbed due to a decrease in the function of the sarcoplasmic reticulum (SR). We studied the differences in the SR function in patients with rheumatic and coronary heart disease, against the background of amiodarone. Cardiac preparations from the atrium of 21 patients with coronary artery disease (CAD) and 14 patients with rheumatic heart disease (RHD) were used in this study. Myocardial strips perfused with oxygenated Krebs-Henzelait solution without and with amiodarone (1 mM/l) at 37°C. The steady state stimulation rate of the muscle strips was 0.5 Hz. The single extraordinary impulse was given as 0.2-1.5 sec after the steady state beat. Then, the first beat after a 4- to 60-sec rest period was evaluated. The extrasystoles of the myocardium in both groups, after long intervals, were decreased after amiodarone treatment. The amplitude of post extrasystoles of amiodarone-treated myocardium showed differences only after long intervals in both groups. Two types of inotropic responses of a failing myocardium after rest periods were observed. Type I post-rest contractions maintained the steady state amplitude after all rests. However, type II was characterized by a reduction in the amplitude of the contractions. Amiodarone treatment of the myocardium showing type I reactions led to an increase in the potentiation after rests, but showed no effect on the reaction of the muscle with the type II response. The results suggested that SR dysfunction was different in CAD and RHD. The realization of the therapeutic effect of amiodarone was found to be dependent on the functional activity of the SR. IJBM 2012; 2(1):9-15.

Key words: *rhythmoinotropic dependence, muscle strips, ischemic and rheumatic heart diseases, amiodarone.*

Introduction

Myocardial remodeling due to chronic heart disease and the subsequent development of heart failure are accompanied by a decrease in the functionally active cardiomyocytes. These cells undergo significant morphological and metabolic reorganization, whose direction and evidence can become essential factors in determining the development of heart failure [1-3]. Depression of the contractile function of the myocardium is associated primarily with a disturbance in the intracellular calcium ion homeostasis [4-7]. This is revealed by a decrease in the myofilament Ca2+ sensitivity accompanied by a significant suppression of the functional activity of the energy-dependent Ca^{2+}-transporting systems [5-10]. A change in the Ca^{2+} homeostasis in the cardiomyocytes determines, to a large extent, the appearance of disordered heart rhythm [11, 12]. The sarcoplasmic reticulum (SR) is known to play a key role in the regulation of intracellular Ca^{2+} transport, as well as provide electromechanical coupling in the cardiomyocytes [13, 14]. Therefore, the contractility of the cardiomyocytes, to a great degree, depends on the

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functional state of this structure. A decrease in the systolic calcium level during heart failure is largely connected with a decrease in the deposition properties of the SR of the cardiomyocytes [15, 16]. It has been experimentally proved that the depression of the SR functions, to a great extent, can be associated with the decreased expression of Ca^{2+}-ATPase protein and ryanodine receptors [17-19]. These changes are reflected in the process of electromechanical coupling and, consequently, influence the chronoinotropic abilities of remodeling myocardium [20-23].

The reaction of the myocardium to changes in the modes of electrical stimulation is known to depend on the functional ability of the SR and energy metabolism within the cardiomyocytes. In the experiment, this fact is used as the basis for the evaluation of the SR state as the main intracellular Ca^{2+} store [24, 25]. Significant distortions of the chrono-inotropic reaction of the myocardium were seen to appear during chronic heart failure [21, 22]. However, at present, the available comparative data on the state of intracellular Ca^{2+} homeostasis during heart failure of different genesis is insufficient.

To treat and prevent heart rhythm disorders developing against the background of heart failure and in the early postoperative period after surgical correction of coronary insufficiency and rheumatic heart diseases the Class III antiarrhythmic drug, amiodarone, is widely used due to its high efficiency and low proarrhythmogenic activity [26-28]. The preliminary results obtained in our study on rat myocardium prove that amiodarone can essentially modify the chronoinotropic response of the myocardium because of its ability to affect the intracellular transport of calcium ions [21-22]. However, this ability during heart failure of different genesis has not been studied practically.

The aim of this study, therefore, was a comparative evaluation of inotropic responses of isolated myocardium of patients with coronary and rheumatic heart disease, after short-term rest periods, against the background of amiodarone.

Methods

Patients: The study was performed on the right atrial trabeculae isolated from patients at the time of surgery of aortocoronary bypass or replacement of aortal or mitral valve. The first group of patients included 21 patients with coronary artery disease (CAD) with exertion angina of functional class III or IV according to the NYHA, with multivessel injuries of the coronary arteries. The mean patient age was 50±2 years. The patients underwent direct surgical revascularization of the myocardium, of two or three coronary arteries. The second group included 14 patients with rheumatic heart disease (RHD). Mitral valve replacement was performed in seven patients, and aortal valve replacement in the other seven. The mean age of the patients in this group was 47±3 yrs. Ejection fraction of all the patients was 49.0±17.3%. All operations were performed using the heart-lung (HL) apparatus and general hypothermia. A fragment of the right atrial appendage was excised while connecting to the HL apparatus. The duration of the disease from the time of registration was 8 to 10 years; concomitant disease was present for a minimum of five years. No significant differences were recorded in any of the clinical parameters in this study, between the two patient groups. All the CD and RHD patients received the standard treatment with antianginal and antihypertensive preparations. The study was approved by the Ethical Committee of the Institute of Cardiology of the Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russia.

Muscle strip preparation: Trabeculae were isolated from the right atrial appendage with dimensions of not more than 1-mm in cross-section and not more than 5 mm in length. The contractility of the muscle was recorded in isometric mode. To achieve this, one end of the muscle was fixed to the wall of a thermostatic flow-through chamber while the other end was attached to the rod of an isometric sensor (a mechanoelectrical transducer). The muscles were perfused with Krebs-Henseleit solution of the following composition (in mM): ***NaCl***, 120; ***KCl***, 4.8; $CaCl_2$, 2.0; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NHCO_3$, 20.0; and glucose, 10.0. Then, Carbogen (95% O_2 and 5% CO_2) was used to oxygenate the solution. Muscle stimulation was performed with 5-ms rectangular electrical impulses applied to two massive silver electrodes located in the perfusion chamber. The frequency of the basic stimulating pulses was 0.5 Hz. Muscle preparations capable of generating a force of at least one-half of the calibration signal (which is equal to 1 V) to the end of the adaptation period (60 min) were used in the experiments. We recoded the curve of the isometric muscle contractions using the bioelectric potential amplifier (UBF4-03, Russia) and a PC using an original applied software package.

Experimental protocol: The excitability of the sarcolemma was determined from the changes noted in the contraction–relaxation cycle in response to an extrasystolic (extraordinary) stimulus. The ability of the SR to accumulate the additional Ca^{2+} ions entering the myoplasm during an extrasystolic stimulus was determined from the changes in the post extrasystolic contractions (PEC). An extrasystolic contraction (EC) was caused by the single application of the extraordinary electrical pulse in 0.2-1.5 seconds after the start of the steady state cycle. EC and PEC amplitudes were expressed as a percentage of the regular cycle amplitude. The dependence of the amplitude changes in EC and PEC on the extrasystolic interval duration was analyzed. The test, which involves the influence of the rest periods, is based on comparative estimations of the curves before and after a short interruption of electrical stimulation (a rest period). This test helps to estimate the ability of the SR cardiomyocyte to take up and to release Ca^{2+} during a contraction-relaxation cycle. Therefore, to estimate the calcium ion recirculation, we plotted the curve of mechanical restitution as the contraction amplitude dependence after the rest period on the duration of the rest period. The effect of the amiodarone was estimated by the change of inotropic response after perfusion of the myocardium with a solution containing 1 μM amiodarone (Sanofi-Aventis, France).

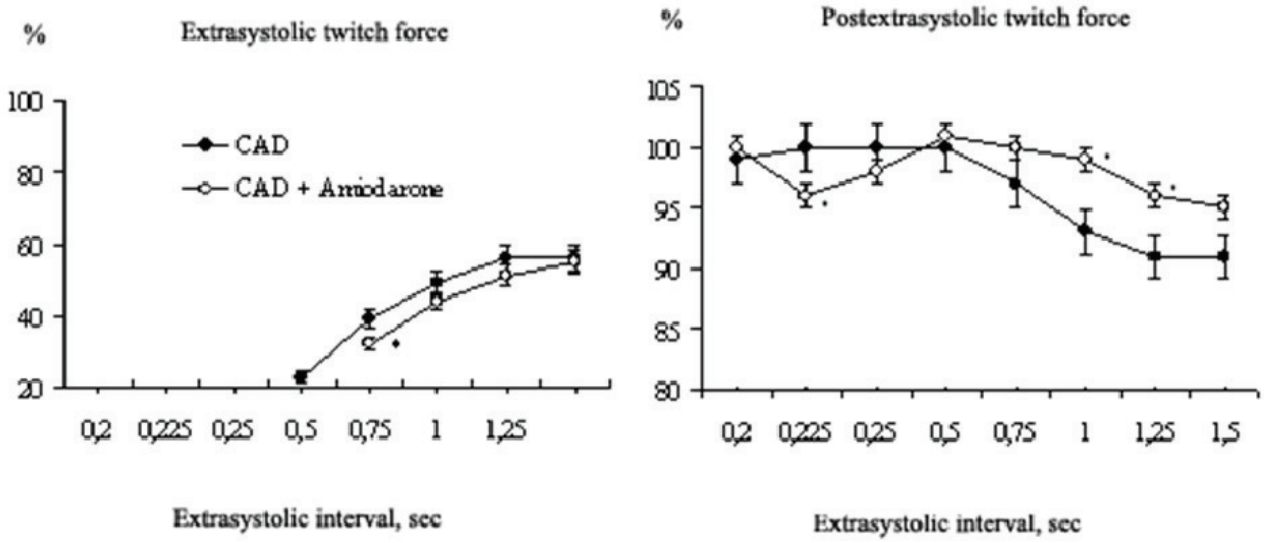
Results and Discussion

The appearance of extrasystolic contractions of the myocardium of patients with coronary artery disease at the time of application of an electric stimulus has been registered only at 0.5 and more seconds (Fig.1). And EC amplitude with an interval of 1.5 secs was found to be only 56.4±4.15% of the basic contraction amplitude. Amiodarone treatment of CAD myocardium showed no principal influence on the intensity of the extrasystolic contractions (Fig.1). An obvious tendency of EC amplitude decrement alone was observed. Simultaneously, an excitability decrease of the remodeled myocardium was observed. Independent extrasystolic contraction occurred only after the extrasystolic impact at 0.75 and more seconds (Fig.1).

The dynamics of post extrasystolic contraction dependency on the duration of extrasystolic intervals after amiodarone treatment of muscle showed visible changes (Fig.1). If at an extrasystolic impact of 0.25 secs the reliability of the PEC amplitude decreased ($p<0.05$), then at long extrasystolic intervals of 1 and 1.25 secs the reliability of the PEC amplitude increased, i.e. the amiodarone was preventing a drop in PEC on a long rests. Therefore, it could be concluded, that amiodarone prevents the depletion of the Ca^{2+} pool in the SR, probably by limiting the leakage current of these ions or making their binding in the SR structure more effective.

On the contrary, during the investigation of the

Figure 1
Amiodarone influence on extrasystolic beat in myocardium of patients with coronary artery disease (CAD).



Note: *Value differs significantly ($p < 0.05$) after amiodarone treatment*

myocardium of patients with rheumatic heart disease (RHD), the amiodarone was found to have no influence on the excitability of the cardiomyocytes (Fig.2). A reliable decrease of extrasystolic contraction amplitude was revealed only with an extrasystolic interval of 1.25 secs. Probably, with such kinds of pathologic changes in the myocardium the amiodarone's poor influence on the structure of the action potential structure, has almost no reflection on the refractory period.

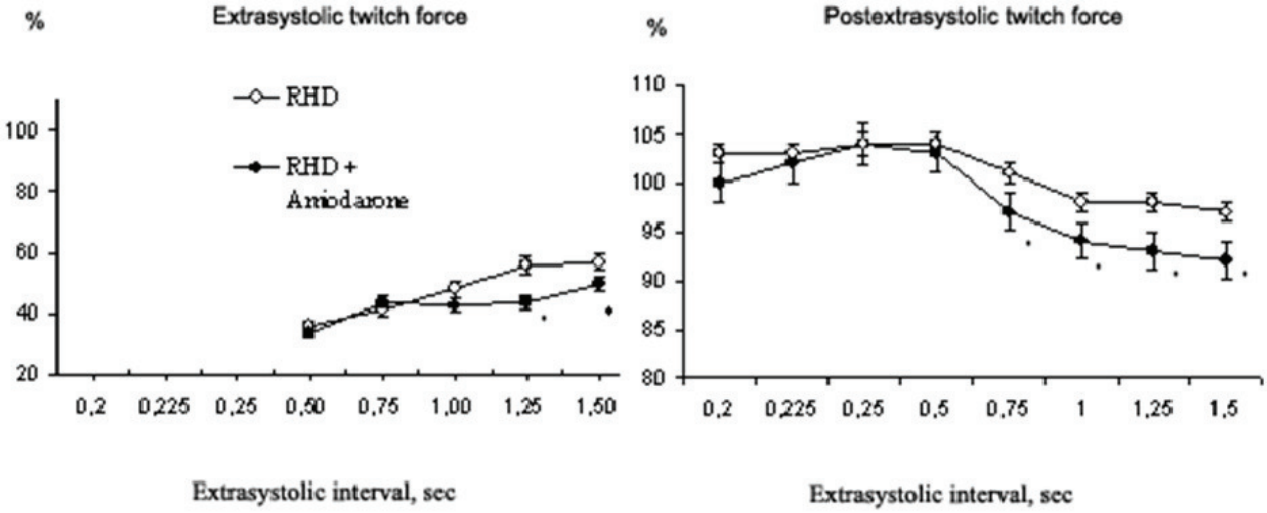
The dynamics of the dependency curve of the postextrasystolic contractions on the duration of the extrasystolic intervals against the background of the amiodarone action shows a notable difference, when compared with the untreated myocardium, on extrasystolic intervals of 0.75-1.5 secs (Fig.2). The amiodarone prevented PEC decay even at extrasystolic intervals of 0.75-1.5 secs. This proves that a greater amount of calcium ions was released from the SR during PEC. As in the case of ischemic myocardium during rheumatic injury, the amiodarone probably provided more effective binding of the calcium ions in the SR, which were then released at the time of the postextrasystolic contraction.

Myocardium inotropic reaction in patients with heart failure on rest periods (post-rest test).

Here it has been discussed that the inotropic reaction of failure of the human myocardium during «post-rest» test could be of two types. At the same time, inotropic reactions of the myocardium with ischemic and rheumatic injury revealed considerable differences. Myocardium strips of the rheumatic heart showed type I reaction characterized by a considerable increase in the inotropic response (Fig.3).

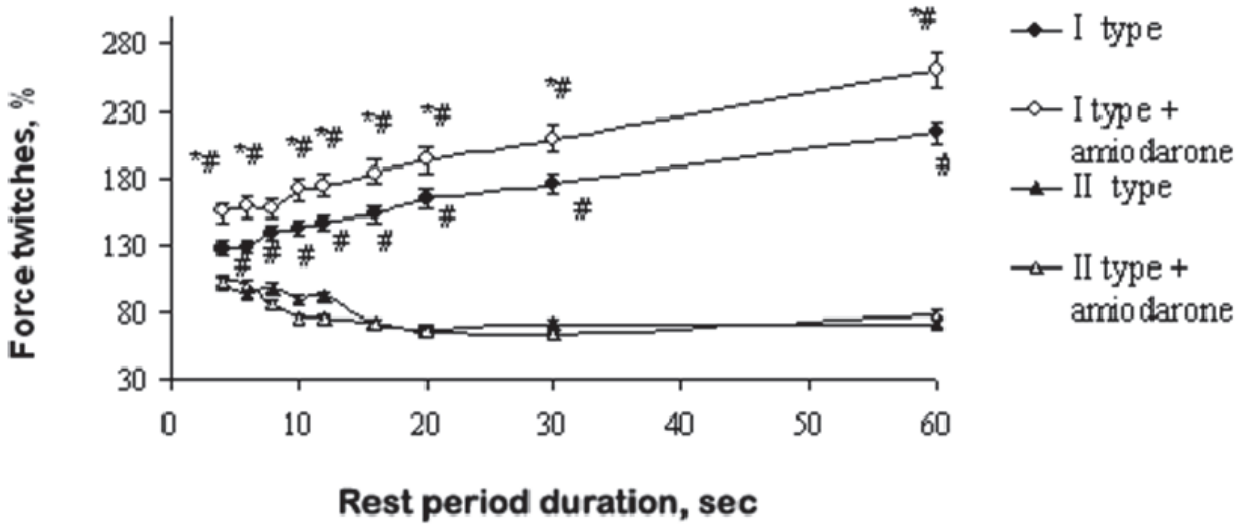
The inotropic response of the myocardium with coronary cardiomyopathy during rest periods having type I reaction did not significantly differ from the basic values (Fig.4). The difference revealed indicates that the type I reaction in RHD is characterized by greater preservation of SR function when compared with ischemic myocardium of the type I reaction. Potentiation of the first contraction after the rest period is realized because of effective Ca^{2+} reuptake during the muscle resting period [28-30]. The results obtained from this study allow us to suggest, that in humans with an ischemic lesion of the myocardium, an activity or quantity of Ca^{2+}-ATPase in the SR is seen to

Figure 2
Amiodarone influence on extrasystolic beat in myocardium of patients with rheumatic heart disease (RHD).



Note: *Value differs significantly ($p < 0.05$) after amiodarone treatment*

Figure 3
Mechanical restitution in patients with rheumatic heart disease (RHD) after amiodaron treatment.



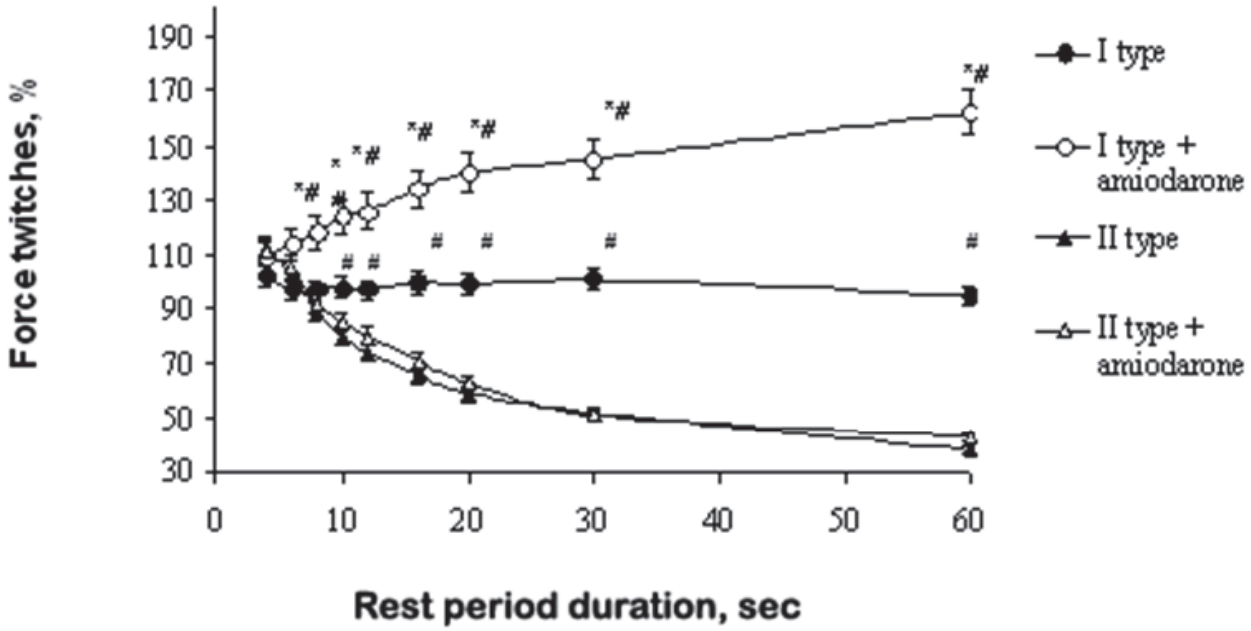
Note: ** - Value significantly differs ($p<0.01$) between groups with the type I reaction before and amiodarone perfusion;*
 # - Value significantly differs ($p<0.01$) between reactions of type I and type II.

significantly decrease. This fact correlates with the data on the decrease in the SR Ca^{2+}-ATPase expression during the genesis of coronary cardiomyopathy [31, 32].

The study of the human myocardium with the type II inotropic reaction was characterized by a decrease in the contraction amplitude after a rest period in relation to the basic contractions. Independent of the heart pathology, with any increase in the duration of the rest period there was an increased step-by-step depression of the contraction response (Fig 3, 4).

However, differences in the manifestation of the type II reaction were noted in the pathologies observed. Contraction depression after the rest periods in the myocardium with rheumatic injury was essentially less than in the heart with coronary insufficiency. Therefore, an amplitude decrease in CD patients after 60 secs was more than 60% (Fig.4), and in the muscle strips of the heart with rheumatic injury, it did not exceed 30% (Fig.3). On comparing the differences revealed, the suppression of Ca^{2+} reuptake in the SR is evident, besides a prior depletion of this calcium depot during short rest of muscle strips. The last situation could be stipulated by a dysfunction of the SR ryanodine receptors [10, 28]. Ryanodine receptors stimulating Ca^{2+} release from the SR and its dysfunction are characteristic of the cardiomyocytes seen in dilated cardiomyopathy [25, 28, 33]. A combination of these mechanisms, probably, is the most adverse and, found in CD patients with the type II

Figure 4
Mechanical restitution in patients with coronary artery disease (CAD) after amiodaron treatment.



Note: ** - Value significantly differs ($p<0.01$) with amiodarone treatment;*
 # - Value significantly differs ($p<0.01$) between reactions of type I and type II.

reaction. The most expressed response of suppression of contraction during «post-rest» test was observed in the muscle strips of CDs patient heart exactly. Manifestation of this effect increased with an increase in the duration of impact.

While repeating «post-rest» test on the muscles treated with amiodarone, a strict regularity in pattern was revealed. The inotropic response of muscles with the type I reaction was strengthened against the amiodarone background, while the effect of the intensity of the type II reaction remained unchanged independent of pathology to be considered. Thus, it could be concluded, that amiodarone positively influences the SR function, promoting the effective binding of Ca^{2+} inside the SR and prevents the "leakage current" of these ions. In light of the fact that amiodarone does not influence the uptake of calcium ions proves the absence of a positive inotropic action of this drug. It must be noted that in all patients with rheumatic lesions and type II reaction in the muscle strips, long episodes of atrial fibrillation are seen. This also indicates the presence of stable disturbances of intracellular calcium ion homeostasis in the cardiomyocytes.

Conclusion

Remodeling of the myocardium leads, on the one hand, to a reduction in the excitability of the heart muscle, and on the other hand, to an increase in the quantity of free calcium ions involved in the realization of extrasystolic contraction. Also, pathologic remodeling of the myocardium leads to a significant suppression of the calcium-accumulation ability of the SR. At the same time, a splitting in the post-rest reaction implies that a different functional activity of the SR occurred in each group studied. A decrease in the functional activity of the cardiomyocytes in the SR is higher during coronary pathology. The results presented indicate that pathologic remodeling of the myocardium is probably formed in two ways. In the first case, the SR preserves its functional activity and the myocardium is characterized by a better degree of preservation of the potential inotropic abilities. In the second case, the SR functions are essentially suppressed. The functional state of the cardiomyocytes of the SR could play a significant role in the realization of the therapeutic action of the class III antiarrhythmic drug, amiodarone. Our study results showed that during remodeling of the myocardium, in the case of preservation of the SR functions, the amiodarone exerts a maximal effect and, conversely, when the functional activity of the SR is essentially suppressed, the amiodarone effect is practically not observed.

Thus, the results obtained in our study enable us to reveal one more property of the class III antiarrhythmic drug, amiodarone, connected with the modulation of the intracellular homeostasis of calcium ions. That property of the drug is undoubtedly positive and probably enables high effectiveness in the therapy of life-threatening arrhythmias.

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