Effect of Amiodarone on the Dynamics of Mechanical Restitution of Rat Papillary Muscles

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We studied the effect of amiodarone (class III antiarrhythmic drug) on the dynamics of mechanical restitution of rat papillary muscle. Amiodarone produced a weak negative inotropic effect and stimulated potentiation of contractility of the muscle preparation after short-term (4-60 sec) cessation of its electrical stimulation. On the other hand, the time of attaining half-maximum amplitude of contractions after amiodarone treatment did not differ from the control. Analysis of curves presenting the drop of potentiation of muscle preparation contractility after resumption of regular electrical stimulation after 60-sec arrest until attaining a stable level showed that the amplitude returned to the initial level by the 9thcontraction-relaxation cycle both in the control and after amiodarone treatment. Coefficient of the drop of contraction amplitude potentiation was virtually the same in the two groups. Presumably, amiodarone does not modulate calcium-binding capacity of the sarcoplasmic reticulum, but improves Ca retention in the sarcoplasmic reticulum stores.

Key Words: *papillary muscles; contractility; sarcoplasmic reticulum; amiodarone*

Many antiarrhythmic drugs used in medical practice exhibit arrhythmogenic effects, which can be due to their negative effects on electromechanical coupling in cardiomyocytes. A manifestation of electromechanical coupling is the relationship between the force and frequency of contractions [4] which can be described by the mechanical restitution curve; changes in mechanical restitution characterize the process of intracellular calcium recirculation [7,12,14]. One of the most effective modern antiarrhythmic drugs is amiodarone, which, however, can show arrhythmogenic effects [2]. The pharmacological effect of this drug is due to its interactions with potassium channels and β-adrenoceptors of cardiomyocyte sarcolemma [8-10,15]. On the other hand, the effect of amiodarone on intracellular calcium recirculation is little studied.

We investigated the dynamics of mechanical restitution of the rat myocardium after amiodarone treatment.

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MATERIALS AND METHODS

Papillary muscles were isolated from the left ventricles of 12 adult male Wistar rats (150-200 g). The animals were sacrificed by cervical dislocation under light ether narcosis, the thorax was opened, the heart was removed and placed into cold Krebs—Henseleit saline. Muscle preparations (5 mm long, <1 mm in diameter) were placed in a thermostabilizing flow chamber. One end of the muscle was fixed to the chamber wall and the other to the stock of isometric pickup of 6MX1C mechanotrone. The muscles were perfused with Krebs—Henseleit solution containing (in mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, and 10.0 glucose (36.5°C). The solution was oxygenated with carbogen (95% O_2 and 5% CO_2). The muscles were stimulated with 5-msec rectangular electric pulses through two massive silver electrodes situated inside the perfusion chamber. The frequency of stimulation was 0.5 Hz. The experiments were carried out on muscle prepara-

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tions developing an effort of at least of $^1/_2$ calibration signal equal to 1 V by the end of the adaptation period (60 min).

The curve reflecting isometric muscle contraction was plotted. The amplitude of contractions (T), maximum rate of contractions (+T/dt), and maximum rate of relaxation (-T/dt) were calculated. In order to plot the mechanical restitution curve, electrical stimulation was discontinued for 4-60 sec. The mechanical restitution curve was plotted as a relationship between the duration of the pause and amplitude of the first contraction after the pause. The inotropic responses of intact muscles (without drug treatment) and muscles perfused with amiodarone in a dose of 1 μM/liter (Sanofi Pharma) were compared. The amplitude of the first contraction after the pause was expressed in percent of the regular contraction.

The data were statistically processed using Statistica 5.0 software, the significance of the results was evaluated using Wilcoxon test.

RESULTS

Muscle perfusion with amiodarone (10^{-6} M/liter) decreased T (91.00±3.27%, p=0.037) and -T/dt (89.00± 3.94%, p=0.043), slightly decreased +T/dt (92.00± 3.79%, p=0.11), and had practically no effect on contraction-relaxation cycle (102.00±2.33, p=0.33) in comparison with the initial level (100%). These data attest to a decrease of calcium pool in the myoplasm under the effect of the drug. Weak negative inotropic effect of amiodarone seems to be due to blockade of adrenoceptors [1-3].

Inotropic response of muscle strips to cessation of electrical stimulation characterizes the capacity of sarcoplasmic reticulum (SR) to accumulate and store calcium ions [5,11]. The increase in amplitude (potentiation in response to cessation of stimulation) is associated with accumulation of calcium ions in SR during the pause, and the amplitude of the first contraction after resumption of stimulation surpasses the amplitude of the regular cycle [12,14]. In our experiment the amplitude of the first contraction after the pause always surpassed the initial amplitude and this potentiation increased with the duration of the pause (Fig. 1). However, the rate of T increase after long pause markedly decreased, which indicated saturation of SR with Ca ions. The maximum increment of T in muscles after 60-sec pause without drug pretreatment was 87.80± 12.12%. Perfusion of muscle strips with amiodarone enhanced potentiation (Fig. 1). Potentiation surpassed the control (p<0.01) for all studied time intervals. The maximum increase in T was 151.20± 10.61%, which almost 2-fold surpassed the control. On the other hand, the time needed for attaining half-maximum increase

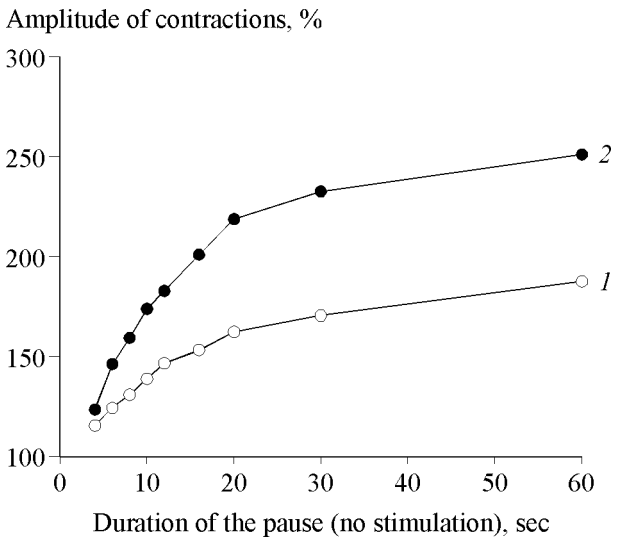

Fig. 1. Mechanical restitution of rat papillary muscles in the control and after amiodarone treatment. Here and in Fig. 2: *1*) control; *2*) amiodarone treatment.

in the amplitude t(T_{50}) virtually did not differ from that recorded after amiodarone treatment and in the control (11.870±0.761 and 10.560± 0.637 sec, respectively), *i.e.*, the rate of Ca ion accumulation in cardiomyocyte myoplasm after amiodarone treatment remained the same as in the control [6]. On the other hand, T potentiation increased after resumption of stimulation in preparations perfused with amiodarone, which attests to massive calcium release from SR during contraction [4]. Pre-sumably, amiodarone promotes more effective "retention" of Ca ions in SR and prevents their "leakage" from SR.

Another characteristic of intracellular calcium recirculation is the decrease in potentiation to the initial level after the pause [12,14]. We revealed that the first contraction after the pause was potentiated, and the

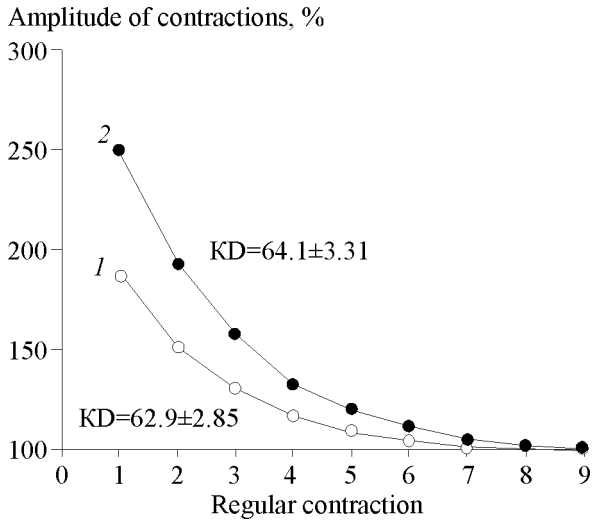

Fig. 2. Decrease of potentiation of papillary muscle after resumption of electrical stimulation after a 60-sec pause.

amplitude of subsequent contraction progressively decreased. The amplitude decreased to the initial level by 9th contraction both in the control and after amiodarone treatment despite the initial difference. T of muscle strips differed in experimental and control preparations ($p<0.01$) in contractions 1-4 (Fig. 2). Plotting of linear regression of the amplitude of the n-th contraction *vs.* the amplitude of contraction $n+1$ helped us estimate the coefficient of the potentiation drop (KD) [12,14], which was 62.90±2.85% in the control and 64.10±3.31% in the experiment (no significant difference). KD reflects recirculation of intracellular fraction of Ca ions [11-13]. Up to 62% Ca ions from the myoplasm are sequestered into SR and 38% are released into extracellular space through the Na^+/Ca^+ exchanger both in control preparations and in muscles perfused with amiodarone [7,13,14]. Hence, amiodarone does not influence Ca-binding capacity of SR.

Therefore, amiodarone can modulate calcium recirculation at the level of intracellular organelles, including SR, by promoting more effective retention of calcium ions in SR and/or preventing their leakage from SR. This fact can be important in amiodarone correction of arrhythmias in chronic coronary patients.

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