PHYSIOLOGY

Effect of Amiodarone on Functional State of Sarcoplasmic Reticulum in Rat Myocardium

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Function of sarcoplasmic reticulum was studied in rat papillary muscles treated with amiodarone. An extra stimulus (0.5 Hz) was delivered to the muscle 0.225 sec after application of a regular stimulus. Postextrasystolic potentiation was evaluated in control myocardial samples and samples treated with amiodarone. The preparation significantly increased all the parameters of postextrasystolic contraction. It was concluded that amiodarone potentiates the ability of sarcoplasmic reticulum to accumulate Ca^{2+} ions.

Key Words: *amiodarone; sarcoplasmic reticulum; papillary muscle; extrasystolic action*

Amiodarone is a highly efficient class III antiarrhythmic preparation [6], which blocks potassium channels and exhibits a number of other properties. It exerts a slight inhibitory effect on α- and β-adrenoceptors [2, 4], exhibits membrane-stabilizing properties via inhibition of inward sodium and calcium currents [8,9]. However, the function of intracellular structures in cardiomyocytes (CMC) against the background of amiodarone treatment is little studied. For example, the effect of amiodarone on calcium-accumulating capacity of sarcoplasmic reticulum (SPR) was carried out only on cultured CMC [12] and pulmonary artery epithelium [11]. However, it is accepted that SPR plays an important role in arrhythmogenesis [5,7].

Our aim was to study the state of SPR in CMC under the effect of amiodarone on rat myocardium.

MATERIALS AND METHODS

The study was carried out on papillary muscle isolated from the left ventricle of male Wistar rats (n=8) weighing 180-200 g. Before the start of the experiment the animals were anesthetized with ether and immobilized by cervical dislocation. The heart was removed and placed in cold Krebs—Henseleit solution containing (in mM): 120.0 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. After excision of the atria, the left ventricular cavity was opened, and the papillary muscle was isolated. The ends of the muscle were tied with kapron snares, which fixed the muscle in a temperature-stabilized chamber. One end of the muscle was fixed to a hook the other was attached to a 6MX1C mechanotron used as an isometric transducer.

The muscle preparations (5-6 mm in length and up to 1 mm in diameter) were perfused with physiological saline bubbled with 95% O_2 and 5% CO_2 mixture (36.0±0.5°C). The muscle was stimulated with rectangular electrical pulses (5 msec) applied via two large silver electrodes. The repetition rate of regular stimulation was 0.5 Hz.

The intracellular calcium homeostasis was disturbed with an additional extra stimulus (extrasystole), which had the same parameters as regular pulses. The extrasystolic stimulus was applied 0.225 sec after regular pulse.

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Excitability of the sarcolemma was accessed by changes in the contraction-relaxation cycle in response to extrasystolic stimulation. The capacity of SPR to accumulate Ca^{2+} excess entering the myoplasm during extrasystolic excitation was assessed by changes in postextrasystolic contraction (PESC) [10].

The experiments were performed on preparations developing no less than $^1/_2$ calibration tension (1 V) at the end of the adaptation period (60 min).

The experiments were performed according to the following scheme: adaptation of the muscle preparations to perfusion regimen and recording of the initial responses to extrasystolic pulses; 10-min perfusion with physiological saline containing amiodarone (10^{-6} M); extrasystolic stimulation.

Muscle responses were digitized and input into a PC. Original software was used to calculate the maximum tension (T_{max}), and maximum (T/dt) and minimum (-T/dt) rates of its changes [1].

The data were analyzed statistically using Statistica 5.0 software and Wilcoxon test.

RESULTS

The extrasystolic stimulus delivered 0.225 sec after regular stimulus induced changes in the shape of isometric contraction curve (Fig. 1): widening of the contraction-relaxation cycle due to appearance of an additional extrasystolic contraction wave.

Extrasystolic stimulus significantly increased (by more than 1.4 times) the total duration of the cycle,

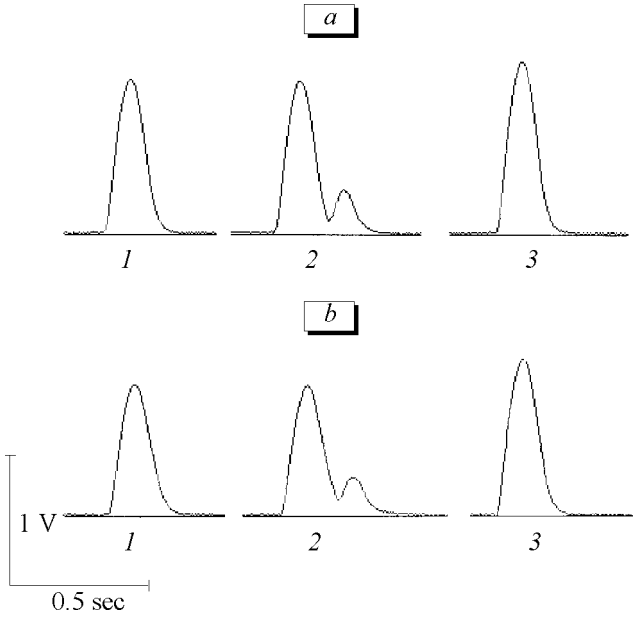

Fig. 1. Typical curves of isometric contraction of rat papillary muscle in control (*a*) and after 10-min perfusion with amiodarone (*b*). *1*) regular contraction; *2*) changes induced by extrasystolic stimulus applied 0.225 sec after the start of regular stimulus; *3*) postextrasystolic contraction.

TABLE 1. Effect of Extrasystolic Stimulus on Parameters of Isometric Contraction (% of Regular Contraction) of Rat Papillary Muscle before and after Amiodarone Application (*M*±*m*, *n*=7)

Parameter	Control	Amiodarone
Amplitude of extrasystolic response	30.00±0.01	25.20±0.02
Duration of contraction-relaxation cycle	144.40±0.04	141.20±0.03

Note. $p<0.01$ compared to regular contraction.

TABLE 2. Effect of Amiodarone on Parameters of PESC (% of Regular Contraction) of Rat Papillary Muscle (*M*±*m*, *n*=7)

Parameter	Control	Amiodarone
T_{max}	119.10±0.02	135.70±0.05*
+dT/dt	118.20±0.01	133.50±0.04*
-dT/dt	123.40±0.04	138.80±0.06*

Note. *$p<0.01$ compared to the control.

the amplitude of extrasystolic contraction being 30.00± 0.01% of regular contraction amplitude (Table 1).

According to modern concept electromechanical coupling [10], the observed changes are related to the entry of extra Ca^{2+} ions from extracellular space into the myoplasm. These ions are stored in SPR, which increases the contribution of SPR into the first postextrasystolic contraction-relaxation cycle. The functional manifestation of this phenomenon is postextrasystolic potentiation of inotropic response of the myocardium (Fig. 1).

The amplitude of mechanical tension developed by the muscle during PESC cycle increased by 19.10± 0.02% in comparison with the amplitude of a regular contraction-relaxation cycle (Table 2).

Application of amiodarone (10^{-6} M) to intact myocardium 1.2-fold reduced the amplitude of extrasystolic contraction. This phenomenon agrees with previous reports on the ability of class III antiarrhythmic preparations to prolong refractory period and decrease myocardium excitability [3]. It can be hypothesized that in amiodarone-treated myocardium the moment of extrasystolic stimulation was closer to absolute refractoriness, which affected the inotropic response of the muscle preparation.

At the same time, amiodarone significantly changed parameters of the first PESC cycle compared to the control (Table 2).

The phenomenon of postextrasystolic potentiation of contractile activity of the myocardium is currently explained by accumulation of extra Ca^{2+} ions by SPR in CMC [8]. From this view, enhancement of postextrasystolic potentiation after amiodarone treatment

can be explained by increased calcium capacity of SPR. The increase in -T/dt observed in our experiments confirms this assumption.

Therefore, amiodarone modifies functional state of SPR by increasing its potency to accumulate Ca^{2+} ions. This effect is most likely directed at activation of SPR membrane transport systems.

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