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ADAPTATION TO STRESS INCREASES RESISTANCE OF THE ISOLATED HEART TO Ca^{++}-INDUCED DAMAGE BY OPTIMIZING CALCIUM PUMP OPERATION IN THE SARCOPLASMIC RETICULUM

F. Z. Meerson, T. G. Sazontova, and I. Yu. Malyshev

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Adaptation to repeated stress prevents or limits disturbances of the electrical stability of the heart and arrhythmia developing in response to stress, ischemia, reperfusion, myocardial infarction, and postinfarction cardiosclerosis [2, 3, 5]. This antiarrhythmic effect is due not only to central stress-limiting mechanisms [5], for it remains largely intact in arrhythmias induced in the isolated heart by large doses of adrenalin [1], or by ischemia and reperfusion [4]. Consequently, during adaptation to stress, a sufficiently effective mechanism of limitation of arrhythmias is formed at the heart level. When this mechanism is studied, it must be recalled that as a result of the action of arrhythmogenic factors (ischemia, reperfusion, catecholamines) the inflow of Ca^{++} into the sarcoplasm is increased [6, 7].

The aim of this investigation was to assess the effect of preliminary adaptation to stress on activity of the calcium pump in the sarcoplasmic reticulum (SPR) and the resistance of the isolated heart to the arrhythmogenic and contractural effects of high Ca^{++} concentrations.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The animals were adapted to stress, which was induced by fixing them in the supine position by their four limbs for 1 h, eight times on alternate days, with three preparatory sessions lasting 15, 30, and 45 min. The control and adapted animals were then heparinized (2000 U/kg) and anesthetized with pentobarbital (50 mg/kg), after which the heart was quickly removed and transferred into standard Krebs–Henseleit solution in a Langendorff perfusion system (11

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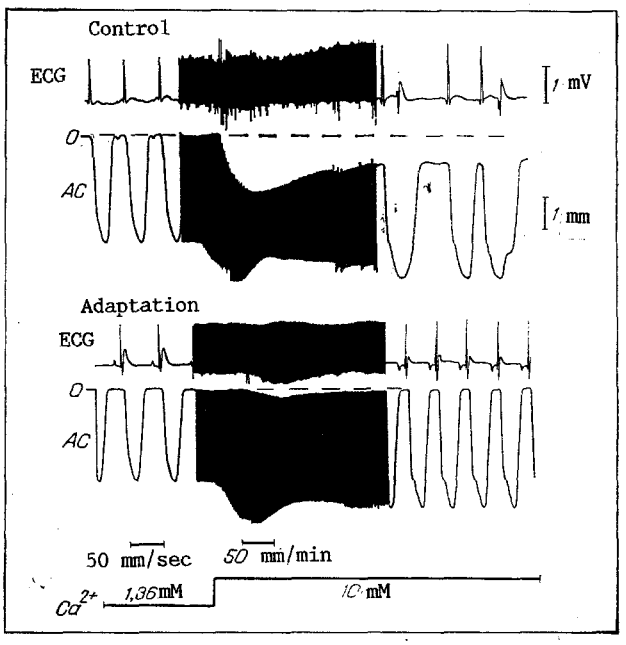

Fig. 1. Effect of adaptation to short periods of stress on electrical and mechanical activity of isolated hearts under the influence of high calcium concentrations. AC (amplitude of contraction) – changes in apicobasal length of heart during contraction. Zero level (broken line) corresponds to complete diastolic relaxation of the ventricles. Time of replacement of normal solution (1.36 mM Ca) by hypercalcium solution (10 mM Ca) shown by step on bottom line.

mM glucose). Mechanical activity of the isolated heart was assessed by means of a TD-112S isotonic transducer, as described by the writers previously [4]. Disturbances of the cardiac rhythm were analyzed in relation to three types of ventricular arrhythmias: extrasystoles, tachycardia, and fibrillation. High Ca^{++} concentrations were created by replacing the perfusion solution with a normal Ca^{++} concentration (1.36 mM) by a solution containing 10 mM Ca^{++}. Changes in pH and osmolarity were not significant and were therefore not compensated. Ca^{++} transport was determined with an "Orion EA 940" ionometer with Ca^{++}-selective electrode, by determining the rate of assimilation of added Ca^{++} by vesicles of SPR. Accumulation of Ca^{++} by mitochondria was prevented by NaN_3, and assimilation of Ca^{++} by sarcolemmal vesicles was virtually absent because of the addition of K^+ oxalate, which does not pass into these vesicles. The hearts were homogenized with an "Ultra-Turrox" homogenizer with 25N-10 blade for 30 sec at speed 8, in medium containing 100 mM KCl, 20 mM imidazole, pH 7.8, and 25% glycerol; the ratio of tissue to medium was 1:4. The rate of Ca^{++} transport was determined in thermostatically controlled cells with mixing for 5 min, with the addition of 50-200 µl of homogenate to 5 ml of medium containing 100 mM KCl, 15 mM K^+ oxalate, 20 mM HEPES, pH 7.0 at 37°C, 4 mM $MgCl_2$, and 5 mM NaN_3. ATP and Ca^{++} were added immediately before determination up to final concentrations of 4 mM and 2-20 µM, respectively. The Ca^{++} concentration in the ATP did not exceed 0.002%. The kinetics of the decrease in the Ca^{++} concentration in medium, which reflects transport of this ion inside the SPR vesicles, was nonlinear, due to the nonlinearity of the electrode itself. To calculate velocity at the point 1-2 min after the beginning of the reaction, a tangent was therefore drawn to the experimental curve and, taking into account Ca^{++} calibration curves in medium with ATP, the rate of Ca^{++} transport was determined. The results were subjected to statistical analysis by the usual methods and the significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

It will be clear from the ECG (Fig. 1) that an increase in the Ca^{++} concentration by 7.3 times led after 15-20 sec to the appearance of ventricular extrasystoles in the control, whereas they were not present in the adapted animals. When the curves characterizing

TABLE 1. Effect of Adaptation on Contractile Function and Rhythm Disturbance of Isolated Rat Heart under the Influence of High Calcium Concentrations

Parameter and experimental conditions	Calcium concentration, mM	
	1.36	10
Maximal amplitude of contraction, mm		
Control (n = 7)	2.78 ± 0.21	3.59 ± 0.21
Adaptation (n = 7)	2.56 ± 0.17	3.42 ± 0.20
Minimal amplitude of contraction, mm		
Control		2.54 ± 0.19
Adaptation		3.30 ± 0.18*
Contracture, mm		
Control	0	1.04 ± 0.19
Adaptation	0	0.36 ± 0.09**
Heart rate, beats/min		
Control	277 ± 10	291 ± 14
Adaptation	270 ± 7	313 ± 15
Coronary blood flow, ml/min		
Control	9.6 ± 0.7	13.7 ± 1.0
Adaptation	8.7 ± 0.6	13.5 ± 0.7
Extrasystoles		
Total number of extrasystoles		
Control	0	76
Adaptation	0	13
Mean number of extrasystoles		
Control	0	11 ± 4
Adaptation	0	2 ± 1*

Legend. Asterisks indicate significance of differences compared with control: *$p < 0.05$, **$p < 0.01$.

contractile function, recorded under isotonic conditions, are evaluated, it must be recalled that with an increase in the concentration of Ca^{++} its positive inotropic effect is realized first, and only later is diastolic relaxation disturbed and a large, transient contracture develops, accompanied by a considerable decrease in the amplitude of contraction. The positive inotropic effect of the excess of Ca^{++} also was well marked in the adapted animals, but by contrast with the control, the contracture was very slight and the amplitude of contraction was not depressed. It follows from Table 1 that the positive inotropic effect of Ca^{++}, developing before the onset of contracture in the control and during adaptation, was identical and was manifested as an increase of 30% in the amplitude of contraction. At the height of development of contracture, the amplitude of contraction was depressed, but during adaptation it remained high, and was indeed 30% higher than in the control. The main parameter, the degree of contracture, was 3 times less in the adapted animals than in the control. The number of extrasystoles in the case of adaptation was reduced by more than 80%.

Preliminary adaptation of the animals to stress thus reduced by several times the magnitude of the direct contractural and arrhythmogenic effects of an excess of Ca^{++} on the isolated heart and, consequently, the membrane mechanisms of Ca homeostatis in the hearts of these animals have significantly greater capacity than in the control to prevent the damaging effects of Ca^{++}. To test this hypothesis, the state of the calcium pump of SPR was studied.

The curves in Fig. 2 reflect the velocity of Ca^{++} transport into SPR depending on its concentration in the medium. In the control, the velocity of the process increased with an increase in the Ca^{++} concentration, and reached a maximum when its level was 10-20 μM, flattening out on a plateau. After adaptation the rate of Ca^{++} uptake in SPR in the presence of a raised Ca^{++} concentration increased much faster, and at a Ca^{++} concentration of

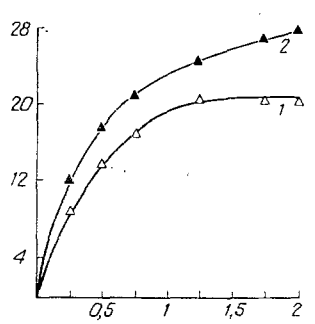

Fig. 2

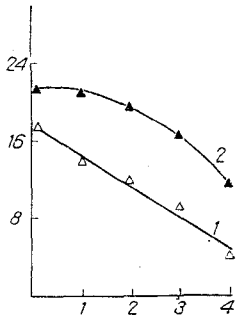

Fig. 3

Fig. 2. Dependence of velocity of Ca^{++} transport into SPR of hearts of control (1) and stress-adapted (2) animals on Ca^{++} concentration in medium. Abscissa, molar concentration of Ca^{++} in medium, $M \times 10^{-5}$; ordinate, velocity of Ca^{++} transport (in mmoles Ca^{++}/min/mg protein).

Fig. 3. Effect of keeping time of heart homogenates of control (1) and stress-adapted (2) animals on velocity of Ca^{++} transport into SPR. Abscissa, keeping time (in days); ordinate, velocity of Ca^{++} transport (in mmoles Ca^{++}/min/mg protein).

5 μM it was one-third higher than in the control. Moreover, the velocity of the process continued to rise in the presence of Ca^{++} concentrations at which in the control it had flattened out on a plateau. This is direct evidence that the ability of SPR in the myocardium of the adapted animals to abolish the excessive overloading of the cells with Ca^{++} was much greater than in the control.

Since the state of the calcium pump depends on its lipid microenvironment, or in other words, on the action of endogenous factors such as lipid peroxidation, phospholipase, etc., whose activity is altered during adaptation [5], we studied the dynamics of inactivation of the calcium pump during keeping, when the action of these factors can be realized. As Fig. 3 shows, SPR of adapted animals is much more resistant to autolysis than in the control. Immediately after homogenization, the velocity of Ca^{++} transport after adaptation was 119% of the control value, and after prolonged keeping it was 290%. The free Ca^{++} level in myocardial homogenates also was sharply increased after adaptation, and the velocity of its accumulation during keeping was slowed by 2.5 times, i.e., leakage of Ca^{++} from its intracellular depots was sharply depressed.

Adaptation to stress thus leads to increased efficiency of function of the calcium pump of SPR and to an increase in the stability of this membrane-bound enzyme toward the action of endogenous damaging factors, and this plays an important role in the cardioprotective antiarrhythmic and anticontractural effects of adaptation.

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