ADAPTATION TO STRESS PREVENTS THE ARRHYTHMOGENIC AND CONTRACTURAL EFFECTS OF THE "CALCIUM PARADOX"

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During adaptation to short periods of stress a sufficiently effective mechanism of limitation of arrhythmia arising during ischemic and, in particular, reperfusion injury is formed at the level of the heart itself [2]. When this mechanism is studied it must be recalled that reperfusion regularly increases the Ca^{2+} inflow into the cardiomyocyte sarcoplasm both from the sarcoplasmic reticulum (SPR) and from the extracellular medium [9], in particular, by a "calcium paradox" mechanism [10]. Data indicating that an excess of Ca^{2+} in the sarcoplasm plays an essential role in the pathogenesis of arrhythmias suggest that the well known cardioprotective effect of adaptation to short, nontraumatic exposures to stress [4] may depend, besides other factors, on the ability to limit the important pathogenetic mechanism of Ca^{2+} overloading, which is associated with the "calcium paradox."

The aim of this investigation was to assess the effect of adaptation to short-term immobilization stress on arrhythmias and contracture of the isolated heart induced by Ca^{2+} overloading in the course of an experimental "calcium paradox."

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The animals were adapted to stress by temporary fixation in the supine position by all four limbs without fixation of the head for 18 days: for 15 min on the 1st day, 30 min on the 2nd day, 45 min on the 3rd day, and 60 min every other day for the remaining period. After the end of the course of adaptation, the control and adapted animals were heparinized (200 U/100 g) and the heart was quickly removed under pentobarbital anesthesia (5 mg/100 g) and connected to a Langendorf perfusion system. A standard Krebs–Henseleit solution (glucose 11 mM) was used for perfusion. The solution was aerated with a gas mixture containing 95% O_2 and 5% CO_2 at 37°C, and at pH from 7.3 to 7.4. The perfusion pressure was 97 cm water. Mechanical activity of the isolated heart was recorded by means of a TD-112S isotonic transducer, fixed to the apex of the heart. Contractural manifestations were assessed as absolute changes in the apicobasal length of the heart during diastole relative to the length at rest, obtained at the end of the 20th minute of the stabilization period of the heart. The ECG and mechanical activity were recorded with the aid of specialized modules of the RM-6000 polygraph. Disturbances of the cardiac rhythm were analyzed with respect to three types of ventricular arrhythmias: extrasystoles, tachycardia, and fibrillation. An experimental scheme in which the normal Ca^{2+} concentration of 1.36 mM was restored to isolated rat hearts after 5 min of calcium-free perfusion served as the model of Ca^{2+} overloading. Very small changes of osmolarity were not corrected; to avoid secondary effects, EDTA was not added to the solution [6].

The results were subjected to statistical analysis by the usual methods, significance of differences between the control and adaptation being estimated by Student's test.

EXPERIMENTAL RESULTS

The curves in Fig. 1 characterize the effect of removal of Ca^{2+} for 5 min followed by restoration of its normal concentration in the solution perfusing the isolated heart: on removal of Ca^{2+} electromechanical coupling ceased in the control and during adaptation. Under these circumstances, evidence of atrioventricular blockade was observed on the ECG in both cases, and

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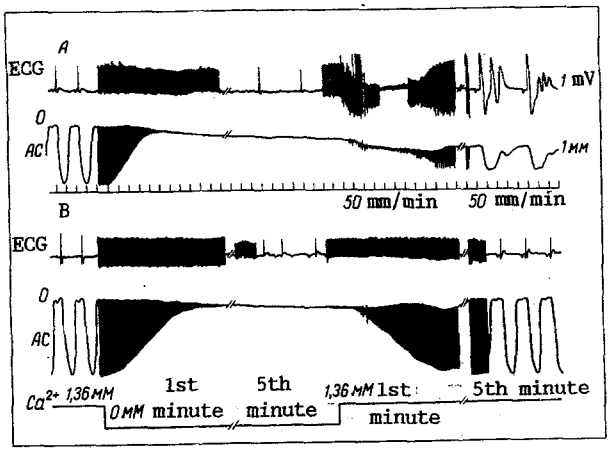

Fig. 1. Effect of adaptation to short exposures to stress on electrical and mechanical activity of isolated heart on restoration of normal Ca^{2+} concentration after 5 min of calcium-free perfusion. A) Control, B) adapted animals. AZ) amplitude of contraction (change in apicobasal length of heart during contraction, 0) zero level (corresponds to complete diastolic relaxation of ventricles). Time of change of solutions indicated by arrows.

TABLE 1. Effect of Adaptation on Contractile Function of Isolated Rat Hearts on Restoration of Normal Ca^{2+} Concentration after Period of Calcium-Free Perfusion ($M \pm m$)

Parameter	Ca^{++} concentration		
	1.36 mM	0 mM, 5 min	1.36 mM, 5 min
Amplitude of contractions, mm			
c	2,63±0,18	0	0,73±0,18
a	2,50±0,19	0	1,53±0,30*
Contracture, mm			
c	0	0,58±0,04	1,09±0,09
a	0	0,41±0,10	0,44±0,19*
Frequency of contractions, beats/min			
c	277±12		237±20
a	269±10		240±7
Control flow, ml/min			
c	9,5±0,6	13,5±0,7	8,5±0,8
a	8,3±0,6	13,4±0,7	7,6±0,6

Legend. Here and in Table 2: asterisk indicates significance of differences compared with control: $p < 0.05$. c) Control, a) adapted animals.

in the hearts of the adapted animals it appeared after a longer latent period (222 ± 30 sec compared with 132 ± 24 sec in the control, $p < 0.05$). The main differences between adaptation and the control were observed on restoration of the normal Ca^{2+} concentration in the solution, when a contracture developed in the control under these conditions, the amplitude of contraction was reduced, and grouped extrasystoles were prominent. All these phenomena were virtually absent in the hearts of the adapted animals in this experiment. The antiarrhythmic and anticontractural effects of adaptation are shown quantitatively in Tables 1 and 2. It follows from Table 1 that, given the same frequency of spontaneous contractions and the same coronary blood flow, after restoration of the normal Ca^{2+} concentration the degree of contracture was 2.5 times less in the adaptation group than in the control, whereas the amplitude of contractions, conversely, was twice as great. The data in Table 2 are evidence that during the first 5 min after restoration of the Ca^{2+} concentration the number of extrasystoles in the experiments with adaptation was

TABLE 2. Effect of Adaptation on Arrhythmia of Isolated Heart Caused by Restoration of Normal Ca^{2+} Concentration in Solution after Period of Calcium-Free Perfusion

Parameter	Group of animals	
	Control	Adapted
Extrasystoles	7	7
Number of extrasystoles	315	175
Total (average, M ± m)	45±7	25±5*
Ventricular tachycardia		
Number of hearts	5	2
Total duration, sec	41	3
Average (M ± m)	5,8±2,0	0,4±0,2*
Ventricular fibrillation		
Number of hearts	2	0
Total duration, sec	18	0

reduced almost by half, and the mean duration of tachycardia was reduced by mopethan 14 times compared with the control. Two cases of fibrillation were observed in the control, but none in the adapted animals.

Thus preliminary adaptation of animals to repeated exposures to stress reduced the contractural and arrhythmogenic effects of the "calcium paradox" by several times.

It has been shown that the main damaging factor in the "calcium paradox" is an intracellular excess of Ca^{2+} [10]. Attention is drawn to changes as a result of which the damaging effects of the "calcium paradox" may be realized and, at the same time, to the possible mechanisms by which adaptation may limit these effects. First, dissociation of Ca^{2+} from the glycocalyx complex during calcium-free perfusion leads to disturbance of the normal structure of the sarcolemma [10]. As a result of this, during the subsequent period of restoration of the Ca^{2+} concentration in the perfusion fluid there is a marked increase in permeability of the sarcolemma for Ca^{2+}. Our experiments prove that, as shown by the parameter of atrioventricular blockade, adaptation significantly increases the resistance of the heart to absence of Ca^{2+} the perfusion solution. This fact suggests that the structural integrity of the cardiomyocyte membranes of adapted animals is at a higher level, and will thus limit the pathogenetic mechanism associated with excessive entry of Ca^{2+} through defects in the sarcolemma during realization of the "calcium paradox."

Second, we know [8] that during calcium-free perfusion Na,K-ATPase activity is reduced. The main result of this depression is as follows: an increase in the intracellular Na^{+} concentration [5] and lowering of the resting potential [11]. This combination of changes has been shown to be arrhythmogenic and contracture-inducing, for it implies activation of Na/Ca-exchange and the possible attainment of the threshold of activation of Ca^{2+} channels, as a result of which the intracellular Ca^{2+} level rises and, as a result of this, the whole range of damaging effects of Ca^{2+}-overloading is realized in the "calcium paradox." Adaptation to stress has been shown to increase the resistance of Na,K-ATPases to damaging influences [4], and probably on this basis it can limit the damaging effects of the "calcium paradox," connected with inhibition of activity of this enzyme.

Third, in experiments on isolated rat hearts a marked increase was found [7] in the *c*AMP content in the cardiomyocytes during calcium-free perfusion. The authors cited [7] concluded that this factor may be involved in the "calcium paradox." We showed previously [1] that adaptation to short periods of stress increases the resistance of the isolated heart to elevation of the *c*AMP level induced by adrenalin. The importance of this effect in the cardioprotective action of adaptation will be determined by the contribution of *c*AMP to the damaging effects of the "calcium paradox," and probably, together with other protective mechanisms, this factor may be involved in the formation of the antiarrhythmic and anticontractural effects of adaptation.

Fourth, a special series of experiments conducted in our laboratory showed [3] that adaptation to repeated stress significantly increases the rate of Ca^{2+} transport in SPR. Under conditions of Ca^{2+}-overloading, the increase in the power of SPR due to adaptation may play a decisive role in the prevention of disturbances caused by the "calcium paradox."

These experimental results as a whole signify that adaptation to short-term damaging exposures to stress increases the resistance of the heart to the pathogenic effect of an excess of intracellular Ca^{2+} and they confirm the view that the antiarrhythmic and anticontractural effects of adaptation in various pathological states may be due, besides to other factors, to the limitation of Ca^{2+}-overloading, arising by a "calcium paradox" mechanism.

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EFFECT OF CARBOCYCLIN ON CARDIAC ATP-ASE ACTIVITY IN NORMOTENSIVE AND STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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It has been suggested that one cause of essential hypertension is a disturbance, for some reason or other, of ionic homeostasis of the cells [5, 6]. Investigations have shown that prostaglandins (PG) play a direct role in the regulation of the electrolyte composition of cells [2, 13]. Prostacyclin (PGI_2) is known to be the principal PG synthesized by the rat heart. In rats with hereditary predisposition to hypertension PGI_2 is formed in smaller quantities than in normotensive animals [4]. An intensive search is currently in progress world-wide for analogs of PG which can be used in medical practice. One stable analog of PGI_2 is carbocyclin (6a-carboprostacyclin) (CC).

The aim of this investigation was to study the effect of CC on ATPase activity in the heart of normotensive Wistar-Kyoto (WKY) and stroke-prone spontaneously hypertensive Okamoto (SHR-SP) rats.

EXPERIMENTAL METHOD

Adult male rats aged 10-12 months had blood pressures of: 125.1 ± 4.2 mm Hg (WKY) and 198.6 ± 5.6 mm Hg (SHR-SP; $p < 0.001$).

BP was measured in the caudal artery by a plethysmographic method using an instrument from "Natsume" (Japan). The animals were decapitated, the heart homogenized in ice-cold isolation medium, and subsequent procedures were carried out in the cold at 2-4°C. Membrane fractions were isolated by fractional ultracentrifugation, using Triton X-100 as detergent. Succinate dehydrogenase activity was measured as the marker enzyme. Its activity in the plasma membrane (PM) fraction was zero, and in

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