the lipid bilayer of the cell membranes and the lesser degree of inactivation of membrane-bound proteins.

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EFFECT OF ADAPTATION TO STRESS ON ELECTRICAL ACTIVITY, CONTRACTILITY, AND RESISTANCE OF PAPILLARY MUSCLE TO EXCESS OF INTRACELLULAR CALCIUM

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KEY WORDS: stress; adaptation; resting potential; resistance

Recent investigations have shown that adaptation of animals to repeated stress increases the resistance of the heart to ischemic, reperfusion, and adrenergic arrhythmias [1, 3]. This protective effect of adaptation has been shown to be largely realized at the level of the heart itself: it is accompanied by increased efficiency of function of the sarcoplasmic reticulum (SPR) and, correspondingly, by increased resistance of the isolated heart to the direct contractural and arrhythmogenic action of high Ca^{++} concentrations [4]. These data make it very probable that adaptation has a wider effect on mechanisms of membrane transport and, as a result, on bioelectrical activity of the cardiomyocytes. However, the question of the effect of adaptation on electrical activity of the cardiomyocytes and its dynamics during calcium loading has not been settled. The aim of this investigation was to study the effect of adaptation of animals to short periods of stress on the resting potential (RP) of the papillary muscle, on its resistance to sodium-deficient contracture, and also on electrical and contractile activity of the papillary muscle during increases in the frequency of contractions and the calcium concentration.

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TABLE 1. Effect of Adaptation to Stress on Electrical Activity of Cardiomyocytes of Papillary Muscle during the Action of an Increasing Frequency of Contraction, and at Normal and High Ca^{++} Concentrations

Parameter	Ca^{++} concentration 2.5 mM			Ca^{++} concentration 18 mM		
	0.5 Hz	1 Hz	3 Hz	1 Hz	3 Hz 5 min	3 Hz 10 min
Overshoot, mV						
control	28,3±1,6	30,0±3,8	14,1±1,9	33,0±3,0	4,3±5,6	5,4±5,8
adaptation	23,4±3,4	21,0±3,2	13,1±2,4	27,7±3,9	13,0±4,4	18,0±5,1
RP, mV						
control	75,3±2,9	77,5±3,0	69,0±1,2	87,5±2,0	75,8±3,0	69,0±5,0
adaptation	79,8±1,1	76,8±1,2	74,3±2,7	83,8±2,8	75,4±3,4	82,0±3,0*
Amplitude of AP, mV						
control	104,4±1,8	107,5±3,4	83,2±4,3	120,3±1,8	80,0±6,6	74,0±8,9
adaptation	103,3±3,5	97,8±3,2	87,4±3,1	111,0±2,4*	88,0±4,0	99,8±4,0*
Duration of repolarization (msec) at undermentioned levels:						
10%						
control	6,0±0,8	5,0±0,7	3,5±0,7	6,2±0,8	1,6±0,2	1,4±0,2
adaptation	7,6±0,6	7,0±0,7	6,2±0,8*	6,7±0,6	3,7±0,8*	2,8±0,3**
30%						
control	15,0±1,6	14,0±1,9	6,2±1,0	11,0±1,0	3,2±0,3	2,5±0,3
adaptation	16,5±1,1	15,7±1,4	12,0±1,5**	12,7±0,8	6,2±1,4*	4,9±0,4**
50%						
control	25,8±3,0	23,4±3,3	9,8±1,5	16,3±1,4	5,2±0,6	3,8±0,4
adaptation	29,3±2,1	30,2±2,7	18,3±2,4**	18,9±0,7	8,8±1,9	7,1±0,7**
70%						
control	81,4±1,6	63,0±12,0	17,7±3,7	29,5±2,9	8,1±0,9	5,7±0,5
adaptation	110,0±16,9	98,0±11,0	38,1±7,9*	47,8±4,0**	13,8±3,0	11,1±1,4**
90%						
control	215,0±16,0	196,0±22,0	50,0±13,0	107,0±24,0	22,1±5,9	13,1±3,2
adaptation	235,0±13,0	220,0±7,0	106,4±18,5*	185,0±12,7*	59,0±13,0*	35,0±5,1

Legend. Significance of differences compared with control: *$p < 0.05$, **$p < 0.01$.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The animals were adapted to repeated stress by fixing them by all four limbs in the supine position for 18 days: for 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min every other day for the remaining period. The papillary muscles were excised from the right ventricles and placed in a chamber containing Krebs–Henseleit solution (11 mM glucose), saturated with carbogen, pH 7.3-7.4, 30°C. The rate of perfusion was 10-12 ml/min. The beginning of the experiment was preceded by stabilization of the muscle from 1 h under isotonic conditions, at L_{max}, and during stimulation by square pulse with a frequency of 0.5 Hz, an amplitude of 1.5-2 threshold values, and a duration of 5 msec. Modulus of the RM 6000 polygraph and the TD-112S isotonic transducer were used for stimulating the muscles and recording their contractile function. The bioelectrical activity of the cardiomyocytes was measured by means of "floating" microelectrodes with 3M KCl solution, MEZ8201 amplifier, VC-9 oscilloscope, and RAT-1100 storage unit ("Nihon Kohden," Japan). A low sodium solution was created by reducing the Na^+ concentration to 9 mM. Sucrose was used as replacement. At the second stage the effect of an increasing frequency of stimulation, in the presence of normal and high Ca^{++} concentrations, on parameters of electrical and mechanical activity of the papillary muscles was studied. The infrequency of stimulation was raised from 0.5 to 1 and 3 Hz in steps; the duration of stimulation at each frequency was 5 min. This series was repeated consecutively with Ca^{++} concentrations 2.5 and 18 mM. The results were subjected to statistical analysis by the usual methods and the significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

The curves of Fig.1 show that toward the 10th minute of action of the sodium-deficient solution, in the absence of stimulation, contracture of the muscles of the adapted animals was 6.1 times less than in the control ($p < 0.01$). The fall of RP under the influence of the sodium-deficient solution in the cardiomyocytes of adapted animals also was less marked: RP at the 10th minute was 69 mV compared with 56 mV in the control.

The papillary muscles of animals adapted to stress were thus more resistant to the action of the sodium-deficient solution, which is known to realize its effect through an increase in the Ca^{++} concentration in the sarcoplasm.

TABLE 2. Effect of Adaptation on Parameters of Contractile Function of Rat Papillary Muscles at Different Frequencies of Stimulation and in Different Ca^{++} Concentrations

Parameter	Ca^{++} concentration 2.5 mM			Ca^{++} concentration 18 mM		
	0.5 Hz	1 Hz	3 Hz	1 Hz	3 Hz 5 min	3 Hz, 10 min
Amplitude of contraction, % of initial length of muscle (control n = 7)	21,8±1,2	20,8±1,4	11,0±2,0	18,0±1,4	4,3±1,1	1,1±0,1
adaptation (n = 7)	19,5±1,0	18,9±0,8	12,8±1,2	17,8±0,9	10,2±2,2*	4,7±1,2*
Contracture, % of initial length of muscle						
control	0	0	0	1,5±1,5	15,4±1,3	28,4±2,1
adaptation	0	0	0	0	4,6±2,3**	12,5±2,8***

Legend. Significance of differences compared with control: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

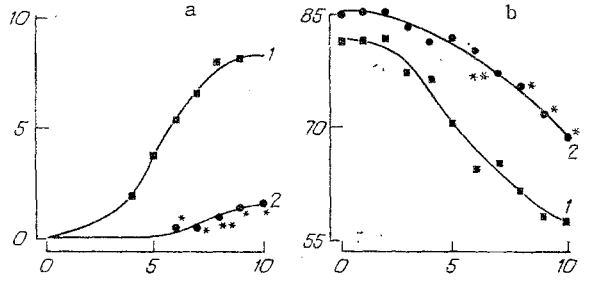

Fig. 1

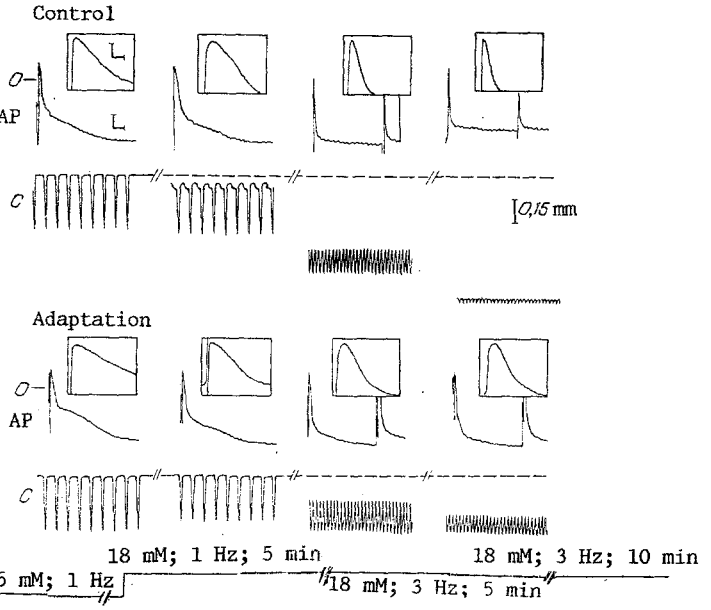

Fig. 2

Fig. 1. Effect of adaptation to short periods of stress on contracture (a) and RP (b) during the action of a sodium-deficient solution on resting papillary muscle. a: Ordinate, contracture (in % of initial length of muscle); abscissa, time from beginning of action of sodium-deficient solution (in min). b: Ordinate, resting potential (in mV); obscissa, time from beginning of action of sodium-deficient solution (in min) . 1) Control; 2) adaptation. Significance of differences compared with control: *$p < 0.05$, **$p < 0.01$.

Fig. 2. Effect of adaptation to short periods of stress on AP and contraction of papillary muscle during combined action of a high calcium concentration and changes in frequency of stimulation. AP) Record of action potential; calibration: 20 mV and 50 msec; inset – apical part of this same potential, calibration: 20 mV and 5 msec. C) Trace of isotonic contraction. Broken line corresponds to initial complete diastolic relaxation. Time of replacement of normal solution (2.5 mM) by solution with high calcium concentration (18 mM) indicated by arrow.

The response of the papillary muscles of adapted and control animals to an increasing frequency of stimulation and to a sharp increase in calcium concentration is illustrated in Table 1 and Fig. 2. Several conclusions can be drawn from Table 1. First, with an increasing frequency of contraction, under conditions of a high Ca^{++} concentration, RP in the control was significantly less than during adaptation and overshoot under conditions of high Ca^{+} concentration and at a frequency of contraction of 3 Hz, the amplitude of the action potential (AP) of the papillary muscles of adapted animals was 25% greater than in the control, and

this must facilitate preservation of a sufficiently high velocity of conduction of the excitation wave under these conditions. Third, the duration of AP with an increase in the frequency of contraction under conditions of a physiological and also a high concentration of Ca^{++} during adaptation was twice as long as in the control (Fig. 2). Thus preliminary adaptation allows an AP of long duration and, consequently, a long duration of the effective refractory period. This shift itself ought to make premature depolarization and arrhythmias less likely. The data in Table 2 and Fig. 2 are evidence that with an increased Ca^{++} concentration the amplitude of contraction of the papillary muscles during adaptation, at a frequency of 180 beats/min, was 2-4 times greater than in the control ($p < 0.05$). This difference, as the data in Table 2 show, is connected with the fact that the contractural effect of an excess of Ca^{++} was 2.5-3 times weaker in the case of adaptation than in the control ($p < 0.01$). In the present experiments, adaptation thus limited contracture of the papillary muscles induced by a low sodium and a high calcium concentration.

The results given above are evidence that the protective effect of adaptation to exposure to stress established previously during adrenergic [1], ischemic, and reperfusion [3] arrhythmias is regularly combined with an antiarrhythmic [4] and anticontractural action of that adaptation and limitation of depression of the electrophysiological parameters during overloading of the heart muscle with calcium, irrespective of whether such overloading is brought about by a combination of a high frequency of contractions and/or a sharp increase in the Ca^{++} concentration during perfusion with a solution with a low sodium concentration. Sodium-deficiency contracture, in the modern view, develops because excessive inflow of Ca^{++} through Na/Ca exchange exceeds the ability of the SPR to take up Ca^{++} [8]. This itself suggested that an adaptive increase in the ability of SPR [4] to take up Ca^{++} plays an important role in the cardioprotective (antiarrhythmia and anticontractural) effect of adaptation and limitation of depression of the electrophysiological parameters. When this phenomenon is analyzed it must be recalled that an excess of Ca^{++} leads regularly to depression of RP, more rapid repolarization, and shortening of the duration of AP, evidently because of activation of the Ca^{++}-dependent outward K^+ current [6]. This combination of changes is arrhythmogenic, for it signifies approximation of RP to the threshold value, shortening of the effective refractory period, and slowing of the conduction of excitation, with a consequent increase in the probability of appearance of ectopic foci, a functional condition block, and the development of re-entry and arrhythmias [5]. It is important to note that depression of RP during calcium overloading is due to the fact that an excess of this cation strengthens the outward K^+-flow and inhibits Na/K-ATPase [7]. This leads to lowering of the transmembrane gradient of K^+ ions and to reduction of the hyperpolarizing current, connected with the sodium pump — the two principal factors in the maintenance of RP.

Accordingly, when the protective effects of adaptation are evaluated, at least two factors must be taken into consideration: first, on account of enhancement of membrane mechanisms for the removal of Ca^{++} and, most important of all, of activity of the Ca-pump of the SPR [4], these changes will be limited and with them, the probability of arrhythmias; second, we know that during adaptation to stress there is a periodic increase in the cAMP and Ca^{++} concentrations acting in the cell. On this basis, the sensitivity of the Ca-dependent K-channel and of Na/K-ATPase to Ca^{++} may be reduced, and this would ensure greater resistance of the membrane potential to an excess of this cation and an increase in the resistance of the heart to arrhythmias. That adaptation can lead to an increase in resistance of Na/K-ATPAse to the action of damaging factors is possible in principle was demonstrated recently by Meerson and co-workers [2].

In the present experiments limitation of depression of RP during adaptation was accompanied by a marked anticontractural effect. This probably means that the protective action of adaptation is achieved primarily through an increase in the power of the SPR and a decrease in the excess of intracellular Ca^{++}, and it is less dependent on reduction of the sensitivity of Na/K-ATPase and of the K channel to Ca^{++} for this last mechanism cannot explain the anticontractural effect. Further support for this hypothesis also is given by our own experimental data, according to which, in the initial conditions with normal Ca^{++} concentration and a frequency of contractions of 0.5 Hz, adaptation did not change RP and AP significantly. The protective effect of adaptation was manifested only when a high frequency of contractions was coupled with the action of high Ca^{++} concentrations.

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