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EFFECT OF ADAPTATION TO INTERMITTENT HYPOXIA ON ELECTRICAL ACTIVITY OF CARDIOMYOCYTES OF THE ISOLATED HEART DURING ISCHEMIA AND REPERFUSION

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UDC 616.127-018.1-02:616.12-005.4/-02:612.273.2

KEY WORDS: adaptation to hypoxia; rat myocardium; action potential; ischemia; reperfusion.

Adaptation to hypoxia not only makes the energy supply to the heart more efficient through an increase in the coronary blood flow [8], an increase in the myoglobin concentration [12], and other metabolic shifts, but also limits adrenergic stressor influences on the heart [1]. As a result of these effects, adaptation to hypoxia has proved to be a powerful antiarrhythmic factor in acute ischemia [5], myocardial infarction [6], and postinfarction cardiosclerosis [4], and also in neurocirculatory dystonia [3]. However, this antiarrhythmic effect of adaptation has not been sufficiently fully explained until very recently, since the effect of adaptation to intermittent hypoxia on electrical activity of the cardiomyocytes has not been studied during ischemia and reperfusion.

The aim of this investigation was to assess the effect of adaptation to intermittent hypoxia on electrical activity of the cardiomyocytes of the isolated heart successively under aerobic conditions during total ischemia, and during subsequent reperfusion.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated heart of male Wistar rats weighing 320-380 g. Adaptation to intermittent hypobaric hypoxia was carried out in a pressure chamber at an altitude of 4000 m for 5 h daily. The course of adaptation consisted of 40 sessions of hypoxia. Contractility and parameters of electrical activity were studied on the isolated heart, perfused by Langendorff's method, as described previously [2], using a TD-112S isotonic transducer and specialized modules of the PM-6000 polygraph (Nihon Kohden, Japan). The transmembrane potential of the cardiomyocytes on the subepicardial surface of the left ventricle was recorded by means of floating microelectrodes [9, 14], filled with

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. N. A. Testemiçanu Kishinev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 574-577, June, 1991. Original article submitted September 12, 1990.

0007-4888/91/0006-0745$12.50

TABLE 1. Effect of Adaptation to Intermittent Hypobaric Hypoxia on Contractile Function of the Isolated Heart during Total Ischemia and Reperfusion (M ± m)

Parameter	Initial parameters under aerobic conditions	Ischemia 5 min	15 min	Reperfusion 10 min	15 min
Heart rate, beats/min					
Control	286±14	90±19	286±17	274±17	268±14
Adaptation	262±12	146±20	306±17	257±20	260±11
Amplitude of shortening, mm, control	2,1±0,12	0,24±0,05	0,55±0,10	0,78±0,10	0,90±0,13
Adaptation	2,0±0,11	0,42±0,06*	0,59±0,12	0,81±0,13	0,95±0,08
Velocity of contraction, mm/sec, control	45,4±3,5	5,0±1,2	9,7±2,4	14,8±2,0	19,5±2,9
Adaptation	43,8±2,3	8,6±1,6	10,6±2,8	15,5±2,7	20,3±2,0
Rate of relaxation, mm/sec					
Control	49,7±3,8	1,9±0,4	13,2±2,5	19,6±2,3	22,2±2,8
Adaptation	44,6±3,6	3,5±0,5*	13,7±3,3	17,2±3,2	23,1±1,7

Legend. Here and in Table 3, seven heart preparations were used in the control series and 10 in the adaptation series; asterisk indicates values ($p < 0.05$) differing significantly from control.

TABLE 2. Effect of Adaptation to Intermittent Hypobaric Hypoxia on Bioelectrical Parameters of Cardiomyocytes of Isolated Rat Heart during Total Ischemia and Reperfusion (M ± m)

Parameter	Initial parameters under aerobic conditions	Ischemia			Reperfusion		
		5 min	10 min	15 min	5 min	10 min	15 min
Resting potential, mV, control	78,6±1,4	65,7±2,5	65,0±1,9	62,4±1,9	64,5±2,0	66,2±2,1	70,6±3,0
adaptation	80,0±0,8	74,4±1,6*	71,6±1,7*	70,1±1,4*	73,3±2,6*	74,9±2,2*	75,8±3,1
Action potential, mV							
control	91,2±0,8	70,6±2,7	70,4±2,3	62,1±2,5	66,4±3,0	69,0±3,9	78,3±4,5
adaptation	92,0±1,8	84,2±4,0*	74,1±1,7	74,5±2,5*	76,2±3,4*	79,0±2,3*	85,6±3,8
Duration of AP, msec, at 50% repolarization							
control	21,0±1,7	20,0±1,8	16,1±1,5	12,5±2,0	15,3±1,5	17,2±1,5	18,3±1,8
adaptation	29,5±2,7*	29,0±2,3*	26,5±2,7*	23,3±2,7*	25,6±2,9*	25,9±3,3*	29,0±3,5*
At 90% repolarization							
control	52,0±2,7	50,5±2,0	38,2±2,3	32,1±2,8	40,2±2,7	46,1±2,3	46,5±3,0
adaptation	63,5±3,5*	60,1±2,9*	48,6±3,1*	47,1±4,8*	55,3±2,9*	58,3±2,8*	60,3±3,4*

3 M KCl solution, and an MEZ-8201 amplifier (Nihon Kohden, Japan). The signal from the output of the amplifier was led to a VC-9 oscilloscope and to an RAT-1100 memory unit (Nihon Kohden) for subsequent analysis. The resting potential (RP), the amplitude of the action potential (AP), and the duration of AP at levels of 50 and 90% repolarization were measured. To record the parameters of AP at the height of the period of total ischemia, when spontaneous electrical activity of the heart had disappeared, short periods of electrical stimulation of the preparations (20-25 sec) by square pulses with a frequency of 0.5 Hz and duration of 5 msec were used, generated by an SEN-3201 stimulator (Nihon Kohden).

In a separate series of experiments the conduction time of the impulse of excitation over the myocardium was determined. In a heart perfused by Langendorff's method the atrium was removed and a constant rhythm of stimulation of 260 pulses/min was imposed on the preparations [10]. To determine the conduction time of the excitatory impulse over the myocardium, bipolar silver electrodes were arranged subepicardially on the surface of the left ventricle [10]. The distance between stimulating and recording electrodes was always constant at 1.5 cm. The electrograms were amplified by means of the biophysical amplifier of the BC-9 oscilloscope and recorded by means of the RAT-1100 memory unit and the automatic writer of a PM-600 polygraph (all instruments were from "Nihon Kohden").

The period of stabilization of the heart under aerobic conditions was 25 min, after which total normothermic ischemia was created by disconnecting the perfusion for 15 min, after which the perfusion was resumed and the function studied during 20 min of reperfusion. The results were subjected to statistical analysis by Student's test.

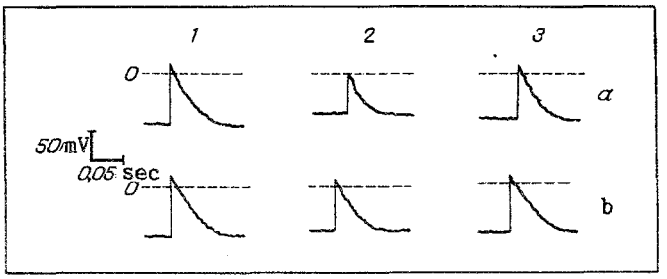

Fig 1. Effect of adaptation to intermittent hypoxia on transmembrane potential of cardiomyocytes of isolated rat heart during total ischemia and reperfusion. 1) Before ischemia, 2) at 15th minute of ischemia, 3) at 15th minute of reperfusion. A) Control, B) adaptation.

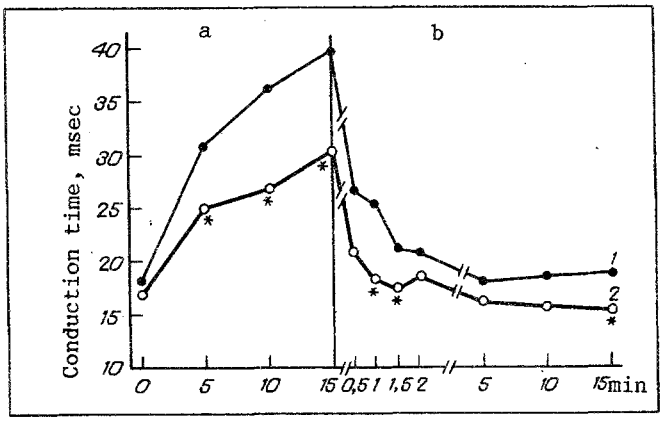

Fig 2. Effect of adaptation to intermittent hypoxia on conduction time of excitatory impulse over left ventricular myocardium of rats. A) Ischemia, B) reperfusion In control (1) and adaptation (2) series nine hearts were used in each case; asterisk indicates significant differences ($p < 0.05$) compared with control.

EXPRIMENTAL RESULTS

The data in Table 1 indicate that under aerobic conditions the contractile function of the heart of the control and adapted animals does not differ. Differences were found at the 5th minute of ischemia, when the amplitude of contraction and the rate of relaxation of the heart of the adapted animals were almost twice as high as in the controls. At the 10th and 15th minutes of ischemia, contractile function was completely absent. During reperfusion, recovery of the contractile function reached 40-50% of the initial level, and differences between the hearts of the control and adapted animals were virtually absent.

Thus as might be expected, preliminary adaptation to hypoxia limited depression of the contractile function of the heart during total ischemia but did not affect recovery of the contractile function during reperfusion.

Taking these data into consideration we assessed electrical activity of the cardiomyocytes in the same hearts during ischemia and reperfusion. The results given in Table 2 reflect the main results of the investigation and they indicate that preliminary adaptation to intermittent hypoxia under aerobic conditions had no effect of RP or AP, but led to a significant increase in AP at different levels of depolarization, i.e., it clearly increased the relative refractory phase. Under ischemic conditions and during subsequent reperfusion the adaptation effect was more marked. Adaptation to intermittent hypoxia, first, inhibited the reduction of RP at all times of ischemia and increased the degree of its recovery during reperfusion. Second, adaptation inhibited depression of the amplitude of AP during ischemia and considerably accelerated its recovery during reperfusion Third and last, preliminary adaptation to hypoxia inhibited the shortening of AP usually developing

during total ischemia, and accelerated recovery of its duration during reperfusion. Examples of AP of the cardiomyocytes during ischemia and reperfusion are shown in Fig. 1. It is generally accepted that lowering the level of RP and the amplitude and duration of AP constitutes the basic complex of arrhythmogenic shifts during ischemia and reperfusion [7, 13]. The fact we established, namely that adaptation to intermittent hypoxia significantly limits all the components of this complex means that the antiarrhythmic effect of adaptation is linked not only with its influence on nervous regulation of the heart and limitation of the adrenergic effect [1], but also with the direct positive action on the mechanisms of ion transport in the membranes and on bioelectrical activity of the cardiomyocytes.

The next stage of the work was to assess the conduction time of the impulse of excitation over the left ventricular myocardium during ischemia and reperfusion. The curves in Fig. 2 show that in the control, toward the 15th minute of ischemia, the antegrade conduction time of the impulse (from the base to the apex of the heart) was more than doubled, but during subsequent reperfusion, it fell quite steeply in the course of 5 min to normal The conduction time in the hearts of the adapted animals increased significantly less — by 14 msec compared with 21 msec in the control. Restoration of the conduction time of excitation took place several times faster than in the control this parameter was within the limits of normal values after only 1 min of reperfusion, compared with 5 min in the control. Similar data were obtained in experiments to study retrograde conduction of the impulse of excitation.

Thus adaptation to hypoxia undoubtedly limits the disturbances of the conduction of excitation in the heart, induced by ischemia and reperfusion, with regard both to intensity and to time. This effect of adaptation evidently also plays a role in its antiarrhythmic action, for it is the conduction disturbance that favors the appearance of such dangerous phenomena as re-entry [11].

On the whole it can be concluded from the facts described above that preliminary adaptation to intermittent hypoxia greatly limits the basic arrhythmogenic disturbances of bioelectrical activity of the cardiomyocytes and the process of conduction of excitation in the heart that are observed during ischemia and reperfusion. This is in full agreement with the fact established previously that this type of adaptation has an antiarrhythmic action in acute ischemia and reperfusion [5], and also with the view that adaptation abolishes disturbances of electrical stability of the heart arising in myocardial infarction [6].

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