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EFFECT OF ADAPTATION TO PERIODIC HYPOXIA ON STABILITY OF MYOCARDIAL ENERGY METABOLISM AND CONTRACTILITY PARAMETERS IN THE PRESENCE OF ACUTE ANOXIA AND REOXYGENATION

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KEY WORDS: adaptation, hypoxia, hypoxic heart damage

Adaptation to periodic hypoxia is a powerful prophylactic factor limiting the onset of arrhythmias associated with acute local myocardial ischemia and reperfusion [1], and also heart damage and the development of arrhythmias in acute myocardial infarction and postinfarction cardiosclerosis [2, 7]. However, no research has been undertaken in order to study the effect of preliminary adaptation to hypoxia on, first, the contractile function of the heart and, second, on parameters of energy metabolism in acute anoxia and subsequent reoxygenation.

The aim of this investigation was to assess the effect of preliminary adaptation to periodic hypoxia on the stability of energy metabolism and contractility parameters of the heart in the presence of acute anoxia and subsequent reoxygenation.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-350 g. Adaptation to periodic hypoxia was carried out in a pressure chamber for 4 h daily for 40 days at an "altitude" of 4000 m. The course of adaptation began from 1000 m, the "altitude" being increased in steps up to 4000 mg by the 8th day. Acute experiments were conducted on the animals under pentobarbital anesthesia (50 mg/kg) under artificial respiration conditions. The rats' hearts were frozen actually in the chest with Wallenberger's forceps for biochemical tests: on the first group of animals in a state of relative physiological rest, on the second group in a state of hypoxia (4 min after disconnecting the artificial respiration), and on the third group during reoxygenation (5 min after resumption of breathing). The frozen hearts were used to determine parameters of myocardial energy metabolism. ATP, ADP, AMP, and lactate were determined with the aid of kits from "Boehringer," and creatine phosphate (CP) by the diacetyl method [3]. Glycogen was solubilized in hot 30% KOH and precipitated with ethanol, then subjected to enzymic hydrolysis by α-aminoglucosidase [4], and the quantity of glucose formed was measured by the glucose oxidase method [6]. Creatine phosphokinase (CPK) activity was determined on a "Hitachi-550" spectrophotometer by the method in [11] with certain modifications. The total and active forms of glycogen phosphorylase were determined by a modified method in [5]. Inorganic phosphorus was determined by the method in [10]. The contractile function of the heart was studied at the same stages of the experiment as parameters of energy metabolism, namely: under conditions of relative physiological rest, during anoxia for 4 min followed by reoxygenation for 5 min. The pressure in the left ventricle was recorded by means of a "Mingograf-35" electromanometer (Elema). The heart rate (HR), systolic and diastolic pressures, and rate of contraction and relaxation of the heart were measured from the pressure curve. The intensity of functioning of structures (IFS) was calculated by the formula ($P \times HR$/mass of left ventricle), where P denotes the pressure developed in the left ventricle.

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TABLE 1. Effect of Preliminary Adaptation to Hypoxia on Stability of Energy Metabolism Parameters in the Presence of Acute Anoxia and Reoxygenation

Parameter	Control			Adaptation to hypoxia		
	initial value	hypoxia	reoxygenation	initial value	hypoxia	reoxygenation
Creatine phosphate, μmoles/g	6.14±0.42 $n=13$	0.60±0.15 $n=13$	4.79±0.49 $n=16$ $p_{1-3}<0.05$	5.58±0.32 $n=13$	0.73±0.13 $n=11$	6.09±0.18 $n=11$ $p_{4-6}<0.05$
Creatine phosphokinase activity, μmoles/g·min	544.0±17.7 $n=11$	460.1±9.9 $n=7$ $p_{1-2}<0.001$	476.5±21.0 $n=8$ $p_{1-3}<0.05$	484.9±18.4 $n=7$	398.0±20.0 $n=8$ $p_{4-5}<0.01$	451.6±15.0 $n=9$ $p_{5-6}<0.05$
ATP concentration, μmoles/g	4.12±0.28 $n=9$	2.84±0.17 $n=7$ $p_{1-2}<0.01$	3.28±0.21 $n=9$ $p_{1-3}<0.05$	3.94±0.25 $n=6$	2.97±0.16 $n=6$ $p_{4-5}<0.01$	3.84±0.21 $n=6$ $p_{5-6}<0.01$
ADP concentration, μmoles/g	0.93±0.13 $n=10$	1.25±0.07 $n=6$ $p_{1-2}<0.05$	0.90±0.09 $n=7$ $p_{2-3}<0.05$	1.07±0.09 $n=6$	1.21±0.04 $n=6$	0.85±0.03 $n=7$ $p_{4-6}<0.05$ $p_{5-6}<0.001$
AMP concentration, μmoles/g	0.23±0.03 $n=8$	0.21±0.04 $n=7$	0.26±0.06 $n=7$	0.18±0.02 $n=6$	0.21±0.03 $n=6$	0.15±0.02 $n=7$
Total phosphorylase activity (A + B), μmoles P/g·min	40.02±1.34 $n=13$	35.82±2.31 $n=13$	31.92±2.72 $n=13$ $p_{1-3}<0.05$	35.61±1.74 $n=14$	33.66±2.34 $n=11$	32.19±2.43 $n=8$
Phosphorylase activity (ratio A/(A + B)	0.18±0.02	0.31±0.03 $p_{1-2}<0.001$	17±0.02	0.21±0.02	0.37±0.04 $p_{4-5}<0.001$	0.15±0.01 $p_{4-6}<0.01$
Glycogen concentration, mg/100 g	407.5±28.9 $n=15$	141.7±15.7 $n=13$ $p_{1-2}<0.001$	152.6±17.1 $n=16$ $p_{1-3}<0.001$	403.8±21.7 $n=13$ $p_{1-3}<0.001$	137.7±14.2 $n=11$ $p_{4-5}<0.001$	147.9±16.8 $n=11$ $p_{4-6}<0.001$
Lactate concentration, μmoles/100 g	1.55±0.11 $n=19$	3.84±0.29 $n=17$ $p_{1-2}<0.001$	3.40±0.41 $n=18$ $p_{1-3}<0.001$	1.03±0.08 $n=9$ $p_{1-4}<0.001$	2.66±0.19 $n=8$ $p_{4-5}<0.001$ $p_{2-5}<0.001$	2.07±0.23 $n=8$ $p_{4-6}<0.001$ $p_{3-6}<0.01$

EXPERIMENTAL RESULTS

The data in Table 1 show that acute hypoxia induced the well known combination of changes in the myocardium of the unadapted animals: a sudden tenfold drop of the CP level, a fall of 30% in the ATP concentration, and an increase in the ADP concentration; CPK activity fell significantly by 16%. Meanwhile the phosphorylase system was activated: there was an increase in the ratio of the active form of phosphorylase to total activity, and a corresponding fall of almost two-thirds in the glycogen concentration and an increase of 2.3 times in the lactate concentration. A tendency was noted for total phosphorylase activity to fall. Toward the end of the 5th minute of reoxygenation the CP and ATP concentrations were not fully restored but amounted to 78% and 80% respectively of the control level. The glycogen concentration and CPK activity were not in general restored, but the decrease in total phosphorylase activity became significant (–20%).

The parameters of energy metabolism studied in the adapted animals in a state of relative physiological rest did not differ from those in the control animals, except the myocardial lactate concentration, which was 33% lower than in the control ($p < 0.001$). The response of the adapted animals to acute hypoxia differed from that of the controls only in that, despite the equal fall in the glycogen concentration, lactate accumulation was one-third less than in the control ($p < 0.001$). A much more definite effect of preliminary adaptation to periodic hypoxia was found during reoxygenation. After 5 min of reoxygenation CPK activity was restored ($p < 0.05$) in the adapted animals and complete restoration of the CP and ATP concentrations was observed, their values being 27 and 17% higher respectively than in the control animals at the same stage of the experiment.

The level of the active form of phosphorylase was significantly lower than initially ($p < 0.01$). Despite no difference in the glycogen concentration at this stage of reoxygenation, the lactate concentration in the adapted animals was 60% of its value in the control series ($p < 0.01$).

Thus preliminary adaptation to periodic hypoxia caused a decrease in the myocardial lactate concentration, and to its maintenance at a lower level during hypoxia and subsequent reoxygenation than in the control; it helped to maintain total phosphorylase activity and to restore CPK activity during reoxygenation, which in turn helped to restore the CP and ATP concentrations.

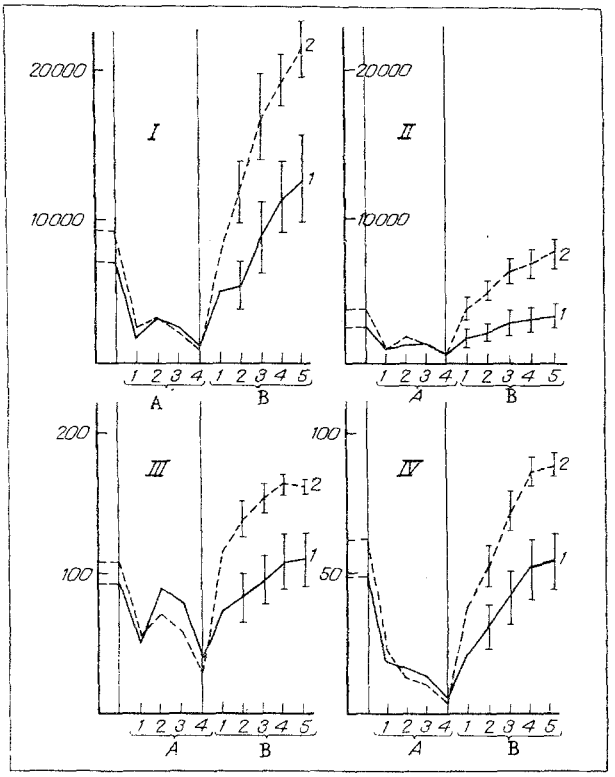

Fig. 1. Effect of adaptation to intermittent hypoxia on stability of parameters of cardiac contractility in the presence of acute anoxia (A) and reoxygenation (B). Abscissa, time (in min); ordinate: I) velocity of contraction of heart (in mm Hg/sec), II) velocity of relaxation of heart (in mm Hg/sec), III) systolic pressure (in mm Hg), IV) IFS (in mm Hg/min·mg). 1) Control, 2) adaptation to hypoxia.

The study of the contractile function of the heart showed that acute anoxia caused depression of its basic parameters in the control and adapted animals to about the same level. However, as the curves in Fig. 1 show, adaptation significantly increased the rate and degree of recovery of contractile function during reoxygenation. In fact, the systolic pressure, velocity of contraction and relaxation, and IFS were restored faster in the adapted animals than in the controls, and with respect to all these parameters, the effect of overrecovery was more marked than in the control. The maximal value of the systolic pressure in the adapted animals during reoxygenation was 51% higher than initially, compared with 21% in the control; the maximal rate of contraction exceeded the original rate by 2.3 times compared with only 81% in the control; the velocity of relaxation by 90% compared with 39% in the control; finally, the maximal value of IFS in the adapted animals was significantly greater than initially ($p < 0.001$), in the absence of any overrecovery effect in the control.

Thus adaptation to intermittent hypoxia significantly increased the rate of recovery of the parameters of energy metabolism and cardiac contractility after acute anoxia.

Some details of the changes discovered in energy metabolism at low a definite opinion to be expressed regarding the mechanism of this phenomenon. In fact, in adapted animals the myocardial lactate concentration was significantly lower in the adapted animals, even under aerobic conditions, but also during anoxia and reoxygenation, than in the controls. This itself may indicate more rapid oxidation of pyruvate in the system of mitochondria, whose efficiency is increased during adaptation to hypoxia [9]. It can also be pointed out that during reoxygenation the content of the active form of phosphorylase in the adapted rats, which rose sharply during anoxia, fell by a greater degree than in the control. On the whole, the situation is evidently that during adaptation to periodic hypoxia, overactivation of glycolysis under conditions of anoxia and reoxygenation becomes excessive due to the increasing efficiency of oxidative phosphorylation. This leads to a greater decrease in the lactate concentration during reoxygenation and reduces competition between H^+ and Ca^+ ions for binding points in the myofibrils [8]. Evidently this explains the more rapid recovery of the contractile function of the heart in adapted animals during reoxygenation.

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TRENDS OF PLASMA CORTICOSTERONE LEVELS IN RABBITS AFTER EXPERIMENTAL CONCUSSION

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The role of the hypophyseo-adrenocortical system (HAS) in the formation of the stress reaction to various stimuli, including photic, acoustic, electrical, and also expectancy, is currently under intensive study [5, 11]. Tissue damage, pain, surgical operations, and anesthesiologic procedures are also known to activate protective and adaptive reactions that determine the course of recovery and repair, and which are expressed as increased synthesis and secretion of ACTH and corticosteroids, processes that can be regarded as the result of activation of the HAS [11, 13].

Since mild head injury (concussion) accounts for the greatest relative proportion of cases of head injury and leads to a significant increase in the degree of disability among the victims, the aim of the present investigation was to determine the plasma corticosterone levels in rabbits at different times after experimental concussion.

EXPERIMENTAL METHOD

Experiments were carried out on 64 male "Gray Giant" rabbits weighing 2-2.5 kg. The animals were kept on the standard animal house diet. Graded trauma was inflicted by the method described previously [9].

Blood plasma was obtained from the unanesthetized animals from 9 a.m. until noon, during decapitation of the animals.

The plasma corticosterone concentration was determined by the method of De Moor et at. [15], with certain modifications. To 50-100 μl of plasma 300 μl of water and 1 ml of hexane were added, the mixture was shaken for 20 sec, centrifuged for 5 min at 1000g, after which the hexane was removed. The residue was treated with 1.2 ml of methylene chloride, shaken, and centrifuged under the same conditions, The extract was washed with 100 μl of 0.1 N NaOH, and then with 100 μl of water, after

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