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EFFECT OF ADAPTATION TO SHORT-TERM STRESS ON RESISTANCE OF PARAMETERS OF MYOCARDIAL ENERGY METABOLISM AND CONTRACTILE FUNCTION TO ACUTE HYPOXIC HYPOXIA AND REOXYGENATION

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Adaptation to short-term stress regularly increases the resistance of the heart to ischemic and reperfusion arrhythmias [3] and limits depression of the contractile function and disturbances of the electrical stability of the heart in experimental myocardial infarction [8]. However, it has not yet been settled whether this protective effect is due purely to limitation of the stress reaction, which is always observed during adaptation to short-term stress [2], or whether this adaptation involves a direct increase in the resistance of the heart to acute hypoxia and subsequent reoxygenation.

The aim of this investigation was to assess the effect of preliminary adaptation to stress on the resistance of the parameters of the energy metabolism and contractile function of the heart to acute hypoxia and subsequent reoxygenation.

EXPERIMENTAL METHOD

The investigation was conducted on male Wistar rats weighing 200-250 g. Adaptation to stress was carried out by immobilizing the animals in the supine position for between 15 min and 1 h, 8 times at intervals of 1 day. Acute experiments were then carried out on the adapted and control animals, under pentobarbital anesthesia (50 mg/kg) and artificial respiration. The rats' hearts were frozen actually in the chest with Wollenberger's forceps: in the animals of group 1 in a state of relative physiological rest, in those of group 2 in a state of hypoxia (4 min after stopping artificial respiration), and in group 3 during reoxygenation (5 min after the resumption of respiration). The frozen hearts were used to determine the parameters of myocardial energy metabolism. ATP, ADP, AMP, and lactate were determined with the aid of kits from "Boehringer," and creatine

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TABLE 1. Effect of Adaptation to Repeated Short-Term Stress on Resistance of Parameters of Cardiac Energy Metabolism to Acute Hypoxic Hypoxia and Reoxygenation

Parameter	Control			Adaptation to stress		
	initial value	hypoxia	reoxygenation	initial value	hypoxia	reoxygenation
CP, μmoles/g	6,14±0,42 (n=13)	0,60±0,15 (n=13)	4,79±0,49 (n=16) $P_{1-3}<0,05$	5,92±0,30 (n=9)	0,56±0,12 (n=9)	7,03±0,42 (n=8) $P_{4-6}<0,05$
CPK, 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) $P_{1-3}<0,05$	522,4±25,4 (n=8)	528,7±28,5 (n=8)	516,7±14,9 (n=8)
ATP, 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) $P_{1-3}<0,05$	4,11±0,30 (n=7)	2,76±0,29 (n=7) $P_{4-5}<0,01$	4,01±0,42 (n=5) $P_{5-6}<0,05$
ADP, 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) $P_{2-3}<0,05$	1,03±0,07 (n=4)	1,17±0,04 (n=8)	1,9±0,10 (n=5)
AMP, μmoles/g	0,23±0,03 (n=8)	0,21±0,04 (n=7)	0,26±0,06 (n=7)	0,18±0,01 (n=4)	0,17±0,01 (n=8)	0,18±0,02 (n=5)
Total (A+B) phosphorylase activity, μmoles P/g·min	40,02±1,34 (n=13)	35,82±2,31 (n=13)	31,92±2,72 (n=13) $P_{1-3}<0,05$	46,68±3,47 (n=9)	44,01±4,01 (n=9)	43,28±3,13 (n=8) $P_{3-6}<0,05$
Phosphorylase activity (ratio $\frac{A}{A+B}$)	0,18±0,02	0,31±0,03 $P_{1-2}<0,001$	0,17±0,02	0,18±0,02	0,37±0,04 $P_{4-5}<0,001$	0,18±0,04
Glycogen, 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) $P_{1-3}<0,001$	502,4±75,5 (n=8)	165,8±38,4 (n=9) $P_{4-5}<0,001$	165,2±34,2 (n=9) $P_{4-6}<0,001$
Lactate, 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) $P_{1-3}<0,001$	1,61±0,40 (n=9)	2,89±0,33 (n=8) $P_{4-5}<0,05$ $P_{2-5}<0,05$	2,21±0,38 (n=8) $P_{3-6}<0,05$

phosphate (CP) by the diacetyl method [4]. Glycogen was solubilized in hot 30% KOH and precipitated with ethanol; enzymic hydrolysis with α-amyloglucosidase [5] followed, and the quantity of glucose formed was measured by the glucose-oxidase method [7]. Creatine phosphokinase activity was determined on a Hitachi spectrophotometer by the method in [10] With some modifications. Total and the active form of phosphorylase were determined by a modified Cori's method [6]. Inorganic phosphorus was determined by the method in [9]. The contractile function of the heart was studied at the same stages of the experiment as the parameters of energy metabolism, namely: at relative physiological rest, 4 min after the creation of hypoxia, and 5 min after reoxygenation. The pressure in the left ventricle was recorded on a Mingograf-34 electromanometer (Elema, Sweden). The heart rate (HR), and rate of contraction and relaxation of the myocardium were measured on the pressure curve. The intensity of functioning of structures (IFS) was calculated by the formula P × HR/mass of left ventricle, where P is the pressure developed in the left ventricle.

EXPERIMENTAL RESULTS

The data in Table 1 show that acute hypoxia caused a well-known combination of changes in the myocardium of the unadapted animals: a sharp fall (by 10 times) of CP, a reduction of one-third of the ATP concentration, and an increase in ADP concentration. Meanwhile activation of the phosphorylase system took place: an increase in the ratio of the active form of phosphorylase to its total activity and a corresponding fall by almost half of the glycogen concentration, whereas the lactate concentration was increased by 2.3 times. A tendency was observed for total phosphorylase activity to fall. By the end of the 5th minute of reoxygenation the CP and ADP concentrations were not yet fully restored but amounted to only 78 and 80% respectively of the control levels. The glycogen concentration and CPK activity were not restored at all, and the fall in total phosphorylase activity became significant (20%).

The response of the adapted animals to acute hypoxia differed from that of the control only in that no reduction of CPK activity is observed, a tendency for total phosphorylase activity to fall was weaker, and despite the fall in the glycogen concentration, lactate accumulation was significantly less than in the control. A much more definite effect of preliminary adaptation to short-term stress was discovered during reoxygenation. In the adapted animals, unlike in the controls, CPK and total phosphorylase activity remained at their original levels, complete recovery of the ATP concentration was observed, and particularly important, the CP concentration after 5 min of reoxygenation was almost 50% higher than in the control animals at the same stage of the experiment. Significant superrecovery was found: the CP concentration was 19% higher than the initial level before

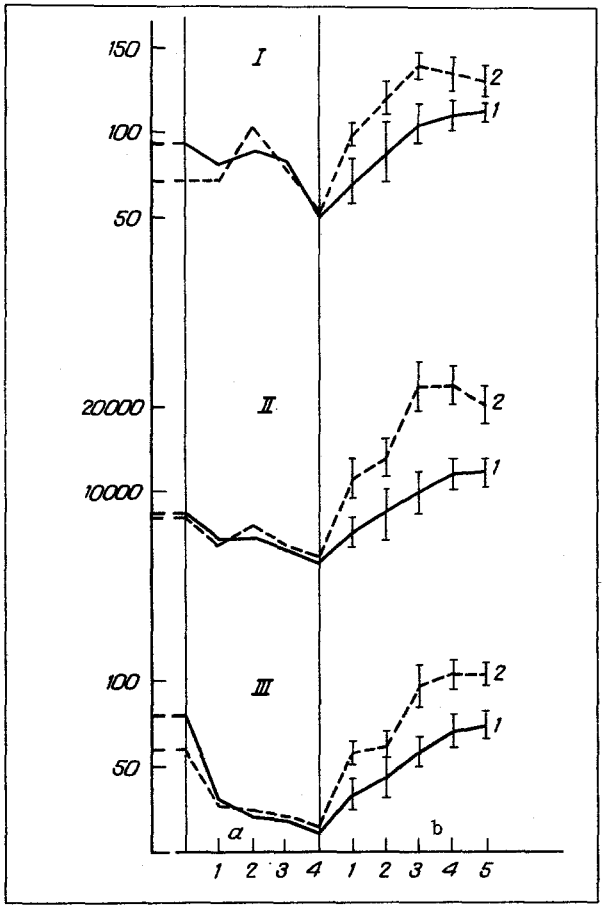

Fig. 1. Effect of adaptation to stress on resistance of parameters of contractile function of the heart to acute hypoxia (a) and reoxygenation (b). Abscissa, time (in min); ordinate: I) systolic pressure (in mm Hg), II) rate of contraction of heart (in mm Hg/sec), III) IFS (in mm Hg/min/mg). 1) Control, 2) Adaptation to stress.

hypoxia. Despite the absence of differences in the glycogen concentration, the lactate level in the adapted animals was more than 50% lower than the control values.

Thus the main effect of adaptation to short-term stress was to ensure maintenance of activity of important enzymes such as CPK and phosphorylase during the damaging action of acute hypoxia, and subsequent reoxygenation, and the superrecovery of CP was evidently a result of this effect.

Physiological experiments showed that acute hypoxia caused depression of the basic parameters of cardiac contractility 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 contractility during reoxygenation. In fact the systolic pressure, rate of contraction, and IFS of the adapted animals were restored faster than in the control, and the effect of superrecovery for all these parameters was significantly stronger than in the control. The maximal value of the systolic pressure in the adapted animals during reoxygenation was 93% above its initial level, compared with only 20% in the control; the maximal rate of contraction was more than three times higher than in the control, compared with one-third as high in the control, and, finally, the maximal value of IFS in the adapted animals was increased by 73%, despite the complete absence of any superrecovery in the control. This is a very important fact, for IFS is the product of HR and the developed pressure, expressed per unit mass of the left ventricle, and it largely determines the energy requirement of the myocardium. Absence of superrecovery, with respect to this parameter, in the control animals and the well-marked superrecovery observed in the adapted animals agree with the fact that only during adaptation, unlike in the control, were superrecovery of CP and full recovery of ATP observed. These data are in agreement with the results of an investigation [1] into the important role of the creatine kinase system and ATP concentration in maintenance of the contractile function of heart muscle.

The result as a whole indicates that adaptation to short-term stress somehow stabilizes the creatine kinase system in acute hypoxia and maintains its ability to restore the CP concentration quickly during reoxygenation; this, in turn, is accompanied by more marked superrecovery of the contractile function of the heart.

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COMPARATIVE CYTOTOXIC ACTION OF QUINOLINIC ACID AND N-METHYL-D-ASPARTATE ON HIPPOCAMPAL NEURONS IN CULTURE

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The neurocytotoxic action of glutamic acid (GA), a CNS neurotransmitter, and its endogenous analog, quinolinic acid (QA), recently discovered by many investigators, laid the foundations for the suggestion that excitatory amino acids (EAA) play a role in the pathogenesis of nervous and mental diseases, accompanied by systemic degeneration of brain neurons [4, 11]. In this connection some relevant experimental investigations have been made of the mechanisms of action of EAA on neurons of various brain structures both in vivo and in vitro.

The excitatory and neurodestructive effect of GA has been shown to be mediated by receptors of three types for its exogenous analogs: N-methyl-D-aspartate (NMDA), kainate, and quisqualate [12]. The results of recent investigations [13] have demonstrated the predominant role of receptors for NMDA in mediation of the neurocytotoxic effect of QA. Meanwhile comparison of the character of the excitatory responses of neurons to QA and NMDA suggested that these responses are mediated by different subtypes of NMDA-receptors [5, 12].

The aim of the present investigation was a comparative morphological study of the cytotoxic effect of QA and NMDA on hippocampal neurons of mouse embryos, developing in cell culture, an object widely used in recent years to study the mechanisms of the neurodestructive action of EAA [4, 9].

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