Superresistance against hypoxia after preliminary adaptation to repeated stress

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Meerson, F., V. Pozharov, and T. Minyailenko. Superresistance against hypoxia after preliminary adaptation to repeated stress. *J. Appl. Physiol.* 76(5): 1856–1861, 1994.—The study investigated the influence of adaptation to stress on resistance to hypoxia. After rats were adaptated to moderate restraint stress, they were anesthetized and exposed to 6% O_2. Adaptation increased tidal volume by 2.6-fold, lung and alveolar ventilation by 1.6- and 1.8-fold, respectively, and O_2 consumption by 1.6-fold; limited lactate accumulation in the liver by 2-fold, in the heart by 34%, in the lung by 36%, and in the blood by 36%; and elevated pH. At the same time, preliminary adaptation to stress inhibited the hypoxic activation of lipolysis and peroxidation in all tissues. The concentration of lipid peroxides decreased after adaptation by 1.3- to 1.5-fold in different organs, whereas the content of free fatty acids diminished by 1.7- to 2.3-fold. Finally, after adaptation, mortality decreased under severe hypoxia by 6.5-fold. Thus, the data suggest that the cross-protective effect of adaptation was achieved by the economization of respiration and circulation, by marked augmentation in the ability of tissues to utilize blood O_2, and by the limitation of processes that are able to damage tissue membranes, namely, acidosis, lipolysis, and lipid peroxidation.

breathing pattern; blood gases and acid-base status; lactate; lipid peroxides; free fatty acids

IT IS KNOWN that adaptation to repeated short-term stress produces wide cross-protective effects. It protects against cold (9), chemical gastric mucous injury (19), and ischemic and reperfusion damage (10) and limits disturbances in myocardial energy metabolism and contracting function during acute hypoxic hypoxia (7). However, the influence of adaptation to stress on respiration, circulation, gas exchange, blood acid-base status, and activity of lipid peroxidation and lipolysis during severe hypoxia associated with high mortality still remains unknown. In the present study we examined the influence of adaptation to repeated mild restraint stress on the resistance against severe hypoxic hypoxia.

METHODS

The investigations were carried out on male Wistar rats. Rats were anesthetized by intraperitoneal injection of α-chloralose (50 mg/kg) and urethan (500 mg/kg). Anesthetics were introduced as a solution of 2 mg/ml of α-chloralose and 20 mg/ml of urethan. The total amount of the introduced solution was 25 ml/kg. Previous investigations have shown that this type of anesthetization produces minimal changes in investigated parameters and stable values for 120–180 min. Acute hypoxic hypoxia was produced by 120 min of inhalation of the gas mixture with 6% O_2.

The rats were subdivided into three groups. Rats of the first group (n = 20) breathed room air for 120 min and were used as control rats. Hypoxic hypoxia was produced in 57 nonadapted rats of the second group. Rats of the third group (n = 21) were adapted to 12 periods of intermittent restraint stress for 24 days. The stress was produced by immobilizing rats in the supine position for 30 min every other day (10). After adaptation, the rats were challenged as described above. There were no significant differences in the body weights of the rats from the different groups. The mean weights of rats were 200 ± 15 (SE) (control group), 196 ± 18 (nonadapted group), and 189 ± 6 kg (stressed group) ($P > 0.05$). To take into account individual differences, the volumetric data were expressed per kilogram of body weight.

We estimated the influence of adaptation on the main factors that determine mortality during acute severe hypoxia. Previous studies performed in our laboratory have revealed that these factors include disturbances of respiration and lung gas exchange, the imbalance between the tissue O_2 supply and requirement, lactate acidosis, the activation of lipid peroxidation and lipolysis, and the limitation of O_2 diffusion through biological barriers (13, 14).

Parameters of breathing and gas exchange were measured using a pneumoresistor, a fast pressure transducer, a mass spectrometer, an analog-to-digital converter, and a computer (17). The pneumoresistor consisted of a three-way tube commonly connected to the airway by means of a cannula after tracheotomy. The cannula and the tube formed the fixed flow resistance of the known pressure-volume characteristics. The pressure drop across the resistor was converted into the deviation of the capacity by the Sylphon and the metallic plate. The capacity deviation was measured by the high-frequency method. Signals from the pressure transducer were connected through the analog-to-digital converter to the computer. The inlet of the tube was connected to a rubber bag containing room air or the gas mixture. To prevent rebreathing, the gas mixture was inhaled from the bag through the tube with the use of a compressor. The volumetric gas flow of the compressor was stabilized at 500 ml/min and was measured by means of A7303 gas mixer tester (Radiometer, Copenhagen, Denmark). The maximum dead space of the pneumoresistor was <0.6 cm^3. The pressure drop across the resistor was proportional to the volumetric gas flow. The pressure-flow characteristic of the resistor and transducer was linear as the gas flow rate was <25 ml/s. To measure concentrations of respiratory gases, the fast mass spectrometer MH-6202 (SZEM, Kiev, Ukraine) was used. The inlet of the capillary of the mass spectrometer was placed into the tube. The amplitude of the gas flow and O_2 and CO_2 concentrations were subsequently sampled at 10 ms, and breath-by-breath data were averaged for each rat every 15–30 s. The computer analysis included the calculation of tidal volume, respiratory frequency, minute volumes of total and alveolar ventilation, O_2 consumption per minute and per single breathing cycle, and partial pressures of O_2 and CO_2 in the inspired and alveolar air. The tidal volume and the expired concentrations of the gases were measured by digital integration of the gas flow and con-

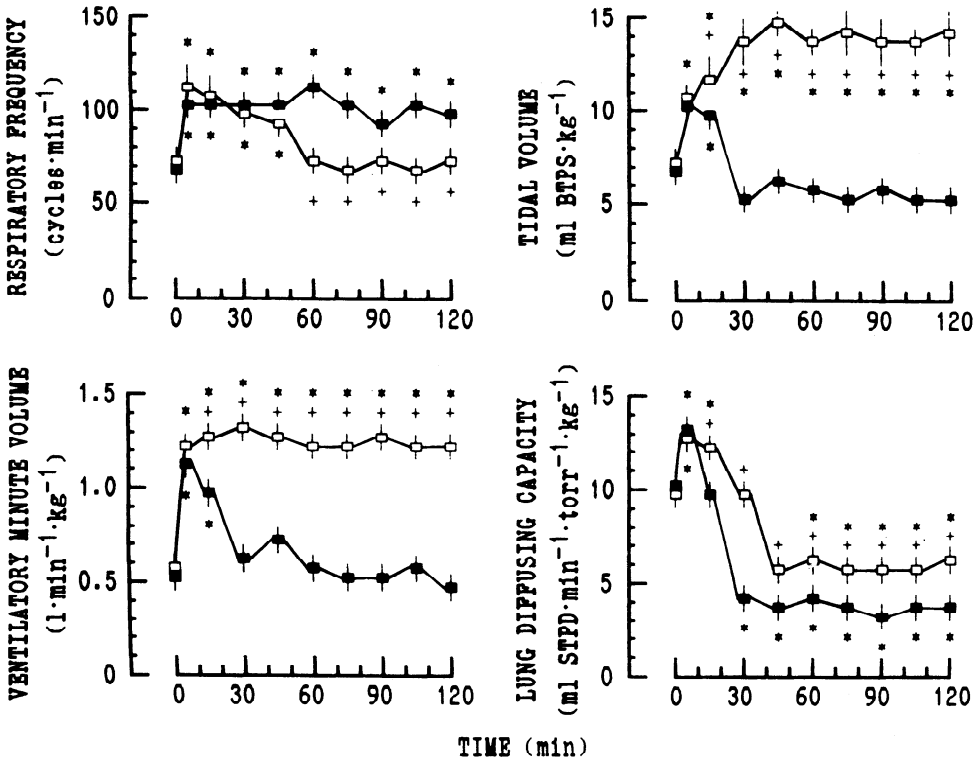

FIG. 1. Influence of adaptation on respiration and O_2 diffusion through biological barriers under severe acute hypoxic hypoxia (6% O_2). Data are means ± SE. ■, Before adaptation; □, after adaptation. * Significantly different compared with control value ($P < 0.05$). + Significant difference between groups ($P < 0.05$).

centrations over the breathing cycle. The end-tidal concentrations of O_2 and CO_2 were used as alveolar concentrations.

Arterial blood was sampled from the right common carotid artery. The internal jugular vein was ligated, and a polyethylene catheter was inserted through the jugular vein and vena cava superior into the right atrium to sample the mixed venous blood. The total amount of the blood sampled during 120 min from a rat equaled 1.2 ml (3 × 0.4 ml). Forty to 45 min were allowed between blood tests in each rat. At any investigated point, blood was sampled from six or more rats of the corresponding group. Preliminary studies in the control group demonstrated that this loss of blood ($\sim$6 ml · kg^{-1} · 120 min^{-1}) does not produce significant changes in the blood hemoglobin concentration and other investigated parameters. Blood gases and pH were analyzed by a blood gas analyzer (Radiometer); hemoglobin concentration was measured using the standard photocolorimetric method. Cardiac output was calculated according to the Fick principle. The balance between tissue O_2 supply and requirement was estimated using the O_2 supply-consumption ratio (SCR) (13, 14). The ratio was calculated by dividing the minute arterial blood O_2 transport by the minute O_2 uptake. Previous investigations (13, 14) indicated that tissue hypoxia develops only when SCR decreases to <2.5 units.

The concentration of lactic acid in the blood and tissue homogenates (brain, liver, heart, muscles) was measured with the enzymatic method (Test Combination 256773, Boehringer, Mannheim, Germany). The level of lipid peroxides was determined by the method of Ohkawa et al. (15), and the values were expressed as nanomoles of malondialdehyde per gram of wet weight, with tetramethoxypropane (Sigma Chemical, St. Louis, MO) used as the external standard. Total lipids were extracted from tissues (3), and the diene conjugation absorption was evaluated by using the ultraviolet spectrum of absorption of the lipid solution in methanol-hexane (5:1) at 233 nm (1). The activity of lipolysis was estimated by measuring the concentration of free fatty acids in the blood and tissues (2).

Diffusing capacity of the lung was used as an index of diffusion through biological barriers and was measured according to the method of Piiper et al. (16). The advantages of the method are the relatively high accuracy and the use of only one level of inspired O_2 concentration. The method is based on measurements of O_2 and CO_2 partial pressures in end-tidal expired air and arterial and mixed venous blood and the rate of O_2 uptake during breathing of the gas mixture with an O_2 concentration of <10–12%. Thus, to determine a control value, rats in the first group inhaled a gas mixture of 10% O_2-90% N_2 for 5–10 min.

Data were analyzed by using analysis of variance for repeated measures. $P < 0.05$ was required for statistical significance. Comparisons between nonadapted and adapted groups were analyzed with the Student's t test.

RESULTS

Data obtained show that adaptation to repeated stress elevates the resistance of rats against acute severe hypoxia. A total of 37 nonadapted rats (65%) died within 120 min of breathing the hypoxic gas mixture containing 6% O_2. After adaptation to stress during a comparable period of the hypoxic exposure, only two rats died (10%). Thus, after adaptation to mild stress the mortality was decreased by 6.5-fold. This finding is especially important because it indicates that adaptation to stress increases the efficiency of all the main mechanisms that are responsible for the adaptation to hypoxia. The following experiments were performed to investigate the possible mechanisms.

An analysis of breathing pattern and lung gas exchange demonstrated considerable differences between control and adapted rats (Fig. 1). In control rats, moderate hypoxic hypoxia increased ventilation, which was maintained during the entire period of hypoxia. During severe hypoxia, ventilation increased only at the beginning of inhalation, followed by a progressive drop to the control value. By contrast, in rats adapted to stress, hyp-

TABLE 1. *Influence of preliminary adaptation to repeated stress on partial pressures in inspired and alveolar air and arterial and mixed venous blood*

	O_2 Partial Pressure, Torr		
	Control	Hypoxia	Adaptation + Hypoxia
Inspired air	146.3±0.7	42.0±0.9*	42.0±1.1*
Alveolar air	101.0±1.4	29.3±1.1*	35.6±0.6*†
Arterial blood	86.7±3.2	28.8±1.4*	34.5±0.5*†
Mixed venous blood	39.7±0.4	14.9±0.8*	16.4±0.2*

Values are means ± SE. Data were obtained after rats breathed ambient air or gas mixture with 6% O_2 for 60 min. * $P < 0.05$ compared with control group. † $P < 0.05$ compared with hypoxia without adaptation group.

oxia increased ventilation during the entire duration of the exposure. In addition to the total ventilation, significant differences in the breathing pattern between the groups of rats have also been found. Nonadapted rats during hypoxia breathed with high frequency and had small tidal volumes. Tidal volume increased only during the first 15 min of hypoxic inhalation. On the other hand, adapted rats demonstrated a progressive increase in tidal volume that was associated with a decrease in breathing frequency. As a result, the tidal volume in this case was increased up to twofold during 60–120 min of hypoxia, whereas tachypnea was never registered.

Deep and slow breathing in rats adapted to stress produced a high efficiency in lung gas exchange. The ratio of alveolar to total ventilation in nonadapted rats under hypoxia equaled 60–70%, whereas in stress-adapted rats it increased to 80–95%. The increase in alveolar ventilation produced a marked increase in alveolar O_2 transport from 20–30 to 60–70 $ml \cdot kg^{-1} \cdot min^{-1}$, i.e., by two- to threefold. Thus, adaptation to mild stress elevates the efficiency of respiration.

During hypoxia, the O_2 difference between air and blood decreased in both groups of rats (Table 1). The difference between alveolar and mixed venous O_2 partial pressures was ~60 Torr, and the alveolar-arterial difference equaled 15 Torr in the control rats. During the time the rats breathed the gas mixture with 6% O_2, these values decreased to 15–20 and 1–5 Torr, respectively. During moderate hypoxia, this decrease usually was compensated for by an increase in lung diffusing capacity. However, data obtained in nonadapted rats indicated that diffusing capacity increased only at the beginning of hypoxic inhalation and then dropped to 40% of the control value (Fig. 1). This disturbance, as well as tachypnea, produced the decrease in O_2 uptake during respiration from 23–25 to 7–8 μl. After the rats were adaptated to stress, diffusing capacity of the lung increased by 1.5- to 2.5-fold compared with that of nonadapted rats. The O_2 uptake per respiratory cycle increased by 1.5- to 2.0-fold, and O_2 pressure in the arterial blood was elevated from 27–29 to 34–35 Torr.

Blood O_2 pressure is only one of the factors that determine the supply of O_2 to the tissue. Hemoglobin concentration and blood flow are the other two important determinants that may play roles in the decrease in mortality after adaptation to hypoxia. However, under acute severe hypoxia the usual compensative increase in hemoglobin concentration was not observed. Moreover, during the initial period of hypoxic inhalation, a significant fall in the hemoglobin concentration and the O_2 carrying capacity of blood was found in the nonadapted rats (Fig. 2). We know of no physiological mechanism to explain this event. One would suppose that this abrupt and substantial fall in hemoglobin content was an artifact or a prob-

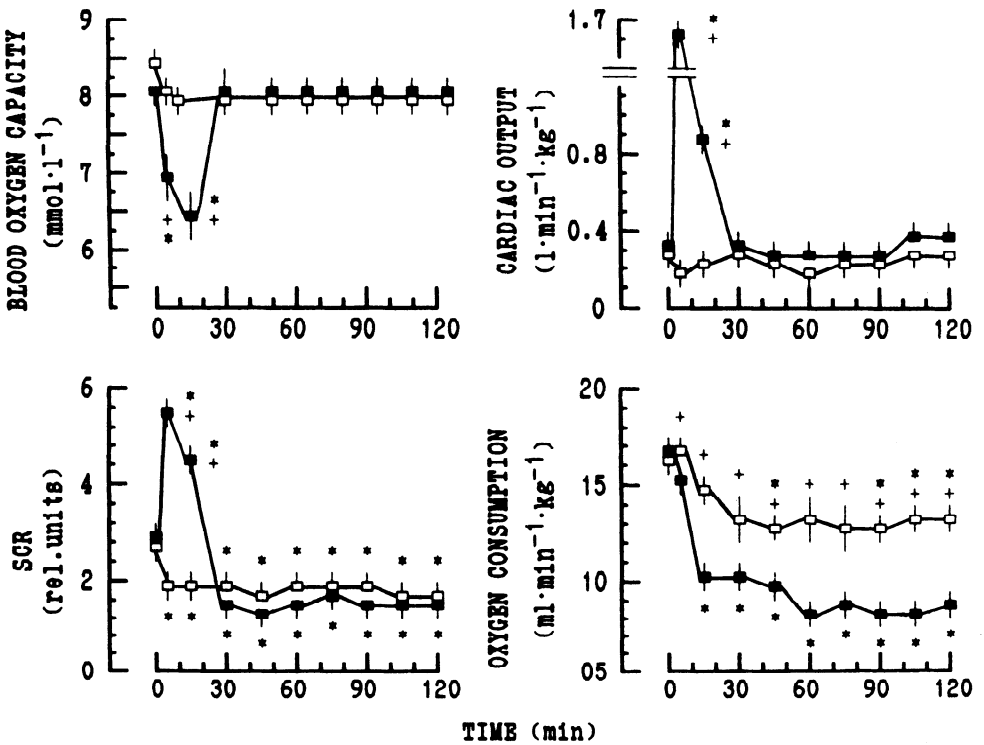

FIG. 2. Influence of adaptation on O_2 supply and consumption under severe acute hypoxic hypoxia (6% O_2). Data are means ± SE. SCR, O_2 supply-consumption ratio. ■, Before adaptation; □, after adaptation. * Significantly different compared with control value ($P < 0.05$). + Significant difference between groups ($P < 0.05$).

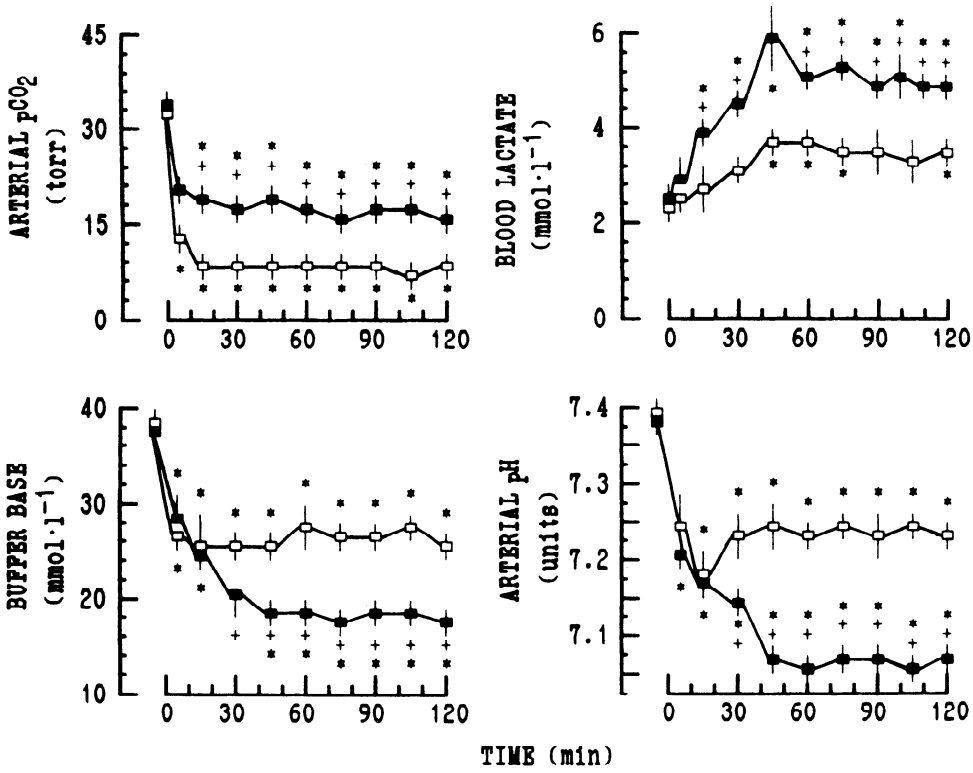

FIG. 3. Influence of adaptation on blood gases and acid-base status under severe acute hypoxic hypoxia (6% O_2). Data are means ± SE. ■, Before adaptation; □, after adaptation. * Significantly different compared with control value ($P < 0.05$). + Significant difference between groups ($P < 0.05$).

lem in blood sampling that led to greater blood loss in this group. However, the blood loss in the rats of different groups was the same (0.4 ml at this point), and it did not produce a decline in the hemoglobin level in the control or stressed rats. The decrease in the hemoglobin level over the first minutes was registered in 28 nonadapted rats that were investigated during the first 15 min of hypoxic exposure. Mechanisms of this event demand, of course, further investigation. Nevertheless, the absence of this decline in the adapted rats may play a role in the increase of resistance to severe acute hypoxia after adaptation to stress.

In nonadapted rats, blood flow was augmented fivefold during the first 15 min of hypoxic exposure (Fig. 2). Nevertheless, increased circulation was not associated with an increase in O_2 uptake or lactate accumulation. On the other hand, the mobilization of circulation during hypoxia was not observed in the adapted rats. Moreover, the O_2 SCR after adaptation to stress decreased to <2 units. Previous investigations have shown that this decrease usually produced an imbalance between tissue O_2 supply and requirement, limited O_2 consumption, and increased tissue hypoxia and lactate accumulation (13). However, data presented in Fig. 2 show an increase in O_2 consumption by 60% in adapted rats compared with nonadapted rats. This increase further resulted in a limitation of the lactate accumulation in the liver by twofold, in the heart by 34%, in the lungs by 36%, and in the blood by 36% (Figs. 3 and 4). The declines in lactate accumulation, hyperventilation, and hypocapnia produced a decrease in hydrogen ion concentration, an increase in bicarbonate concentration, and, finally, an elevation in blood pH (Fig. 3).

Further data analysis showed that severe acute hypoxic hypoxia in nonadapted rats led to the activation of lipolysis and, as a result, to an increase in the concentration of free fatty acids (FFA) in the blood (by 3.5-fold) and in the organs (Fig. 4). Hypoxia activated the next important mechanism of membrane damage, namely, lipid peroxidation (Fig. 4). Adaptation to stress limited the hypoxic activation of lipolysis and peroxidation in all tissues. Indeed, the FFA concentration was diminished by 1.7- to 2.3-fold, whereas the concentration of conjugated dienes was diminished by 1.6-fold in the brain and by 30–40% in the rest of organs compared with corresponding values in nonadapted rats. The concentration of lipid peroxides was decreased after adaptation by 1.3-fold in the heart, 1.2-fold in the lung, and 1.5-fold in the muscles.

DISCUSSION

When the cross-protective effects of preliminary adaptation to repeated moderate stress impacts are being analyzed, the following should be taken into consideration. Under severe acute hypoxic hypoxia, 26 nonadapted rats (45%) died during the first 10–20 min. After adaptation, rats did not die during the first 30 min of the hypoxic exposure. Thus, it may be concluded that adaptation produced protective effects at the initial stage of hypoxia. On the other hand, total duration of the hypoxic exposure may be subdivided into two stages when analyzing the dynamics of parameters investigated in nonadapted rats (see Figs. 1–3). During the first 15–30 min after the beginning of hypoxia in nonadapted rats, an increase in respiration and circulation was registered. This initial increase in the cardiac output produced an increase in the tissue O_2 supply despite the extreme decrease in the

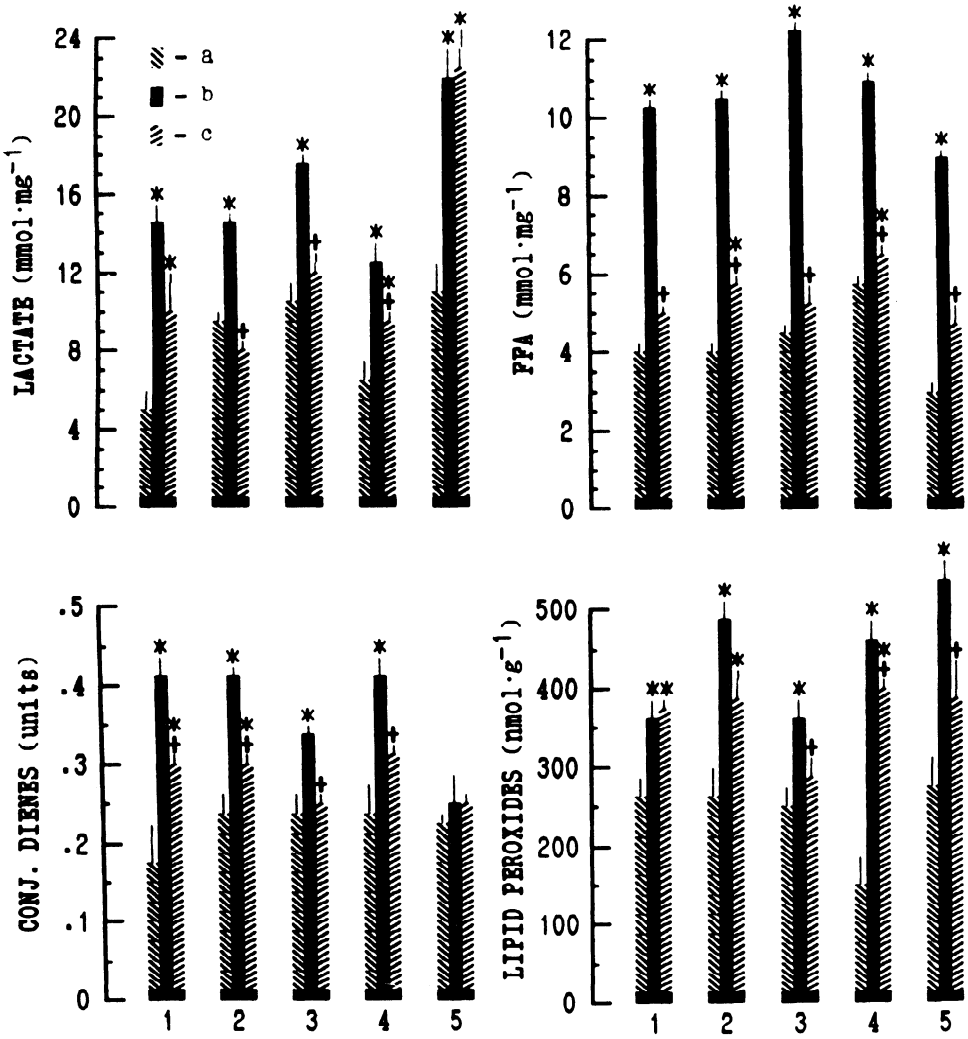

FIG. 4. Influence of adaptation on accumulation of lactate, free fatty acids (FFA), lipid peroxides, and diene conjugation absorption in brain (1), liver (2), heart (3), lung (4), and muscles (5) under severe acute hypoxic hypoxia (6% O_2). Data are means ± SE. * Significantly different compared with control value ($P < 0.05$). + Significantly different compared with nonadapted group ($P < 0.05$). a, Control; b, before adaptation; c, after adaptation.

air and blood O_2 concentrations. However, this strong elevation in the O_2 transport was in fact not helpful, because it did not limit the lactate accumulation in tissues and therefore did not decrease the severity of tissue hypoxia but demanded additional energy expenses. The important result of adaptation to stress was the absence of this expensive reaction of respiration and circulation during the initial, most dangerous, period of severe acute hypoxia. When analyzing this adaptive economization of respiration and circulation, one should take into consideration that adaptation to stress is generally accompanied by the activation of central mechanisms restraining the stress reaction, namely the γ-aminobutyric acid-ergic, opioidergic, cholinergic, and serotoninergic systems (6, 11). It is likely that these systems play a role in the limitation of the ineffective and energy-expensive mobilization of respiration and circulation during the initial stage of hypoxic exposure. It has been shown in our laboratory that severe hypoxia increased the respiratory sensitivity to CO_2 (18). This increase may play a certain role in the economization of ventilation and O_2 transport after adaptation. It should be noted that the economization of respiration and circulation under hypoxia after adaptation to stress was achieved in different ways. A limitation of the energy expense on circulation was maintained by a marked fall in cardiac output, whereas that on respiration was maintained by a shift in the breathing pattern toward deep and slow breathing.

However, the huge decrease in mortality after adaptation to stress may not be completely explained only by the limitation of energy expenses. Adaptation also did not produce an increase in the blood O_2 transport from lung to tissues. Thus, it is likely that the main mechanism of the phenomenon is the elevation in the ability of tissues to utilize blood O_2. This important change has been determined by at least two main factors. The first factor is an adaptive limitation of processes responsible for hypoxic damage, namely, an activation of lipolysis and lipid peroxidation. The second factor is the preservation of O_2 utilization and oxidative phosphorylation in mitochondria under severe acute hypoxia by preliminary adaptation to stress. Recently it was shown that adaptation to repeated restraint stress led to the development of the phenomenon of adaptive stabilization of structures (PhASS) (11). PhASS was accompanied by an increased stability of cytoplasmic membranes and structures of sarcoplasmic reticulum, mitochondria, and nuclei against autolysis and lipid peroxidation. It is likely that under present experimental conditions the development of PhASS should have played a role in the preserva-

tion of cell structures under severe hypoxia and thereby maintained the high level of O_2 diffusion and consumption.

In addition to the direct action of O_2 deficiency, acute severe hypoxia also produced a stress reaction. This stress and the actions of stress hormones might play a role in the development of hypoxic damage. The activation of the aforementioned central stress-limiting systems and some local ones, especially prostaglandin, antioxidant, and adenozinergic systems during adaptation to repeated stress (5, 11), inhibits the damaging action of that accompanying stress. It should also be taken into account that repeated stress impacts the phenomenon of desensitization, decreases adrenoreactivity, and limits an adrenergic blow toward target organs (8, 12).

Thus, the data obtained show that the regulatory economization of O_2 transport and PhASS are two main factors that are responsible for the antihypoxic effect of adaptation to stress. Meanwhile it should be noted that preliminary adaptation changes considerably the reaction of respiration during acute hypoxia. The principal change consists of the following. In adapted rats, severe hypoxia produces high stable hyperventilation, an unexpectedly high loss of CO_2, and marked alkalosis. In fact, CO_2 tension in the arterial blood was decreased to 10–12 Torr (see Fig. 3). On theoretical grounds (4), one would expect the cessation of the rhythmical activity of the respiratory center under this extreme hypocapnia. However, in our experiments that result has not been observed. Mechanisms of this phenomenon appear to be of great importance and should be studied independently.

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Received 24 July 1992; accepted in final form 13 September 1993.

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