Antihypoxic Effect of Adaptation to Stress and of Adaptation to Electrostimulation: a Comparative Study

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In an earlier investigation into adaptation to stress we subjected experimental animals to repeated brief periods of immobilization [1]. It was found that such adaptation developed in the animals super-resistance to sublethal hypoxic hypoxia; it reduced mortality 6.5 times when the experimental animals breathed a gaseous mixture containing 6% oxygen. The important question here is whether the immobilization procedure can be replaced by a milder intervention, one that is more suitable for humans. In recent years transauricular electrostimulation (conducted with a Lasper electroacupuncture device designed for humans) has been found to produce in mice the kind of cardioprotective effect that is obtained by adaptation to stress [6].

The purpose of the present study is twofold. Firstly, it assesses the effect of the passing of an electrical current through the heads of the experimental animals during transauricular electrostimulation on their respiration, circulation, and resistance to hypoxia. And secondly, it compares this effect with that produced by adaptation to stress.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 150-200 g each. In the first series of experiments (n=10) the parameters of the electrical current passed through the heads of the animals (under anesthesia) during transauricular electrostimulation were determined, and the effect of the electrostimulation on respiration and circulation was assessed. In the second series of experiments (n=53), carried out over 10 days, the animals were kept in a vivarium without being exposed to any external influences. In the third series the animals were kept in a vivarium and were subjected daily to transauricular electrostimulation for 15 minutes in a waking state. The electrostimulation was carried out by inserting standard needles designed for facial acupuncture into a certain point of the floor of the auricle. The needles were connected to an electrostimulator (Lasper CS-504, Japan). The electrostimulation lasted 10 minutes on the first day and 20 minutes each day thereafter for the next 9 days. During the first 5 minutes of electrostimulation the animals were held in the hands of the researcher, who detected no signs

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TABLE 1. Effect of Adaptation to Stress and of Adaptation to Electrostimulation on Respiration, Circulation, and Oxygen Transport (90 Exposures)

Parameter	Breathing of atmospheric air control I	Sublethal hypoxia (the breathing of a gaseous mixture containing 6% oxygen)		
		control II	adaptation to stress	adaptation to electrostimulation
Frequency of respiration, cycles/min	74.1±1.6	105.0±8.0	65.6±1.6	98.6±2.42
Respiratory volume for each cycle, ml/100 g×min	52.6±0.05	55.6±0.48	91.1±0.4	57.0±0.13
Minute respiratory volume, ml/100 g×min	52.6±2.2	55.3±6.2	91.2±8.8	56.1±6.2
Minute volume of circulation, ml/100 g×min	33.7±2.0	21.6±8.1	26.2±2.9	55.8±4.2
Pulmonary shunt, %	6.2±0.04	44.56±0.30	22.4±0.40	5.0±0.20
pO_2 in arterial blood, mm Hg	86.7±3.2	23.6±0.5	34.5±0.5	25.7±1.4
pO_2 in mixed venous blood, mm Hg	39.7±0.4	14.9±0.8	16.4±0.2	18.5±0.6
Arterial−venous difference in pO_2	47.0±2.8	8.7±0.3	18.1±0.3	7.2±0.8
Transport of O_2 by arterial blood, ml/100 g×min	6.02±0.03	1.7±0.07	2.4±0.15	3.9±0.17
Uptake of O_2, ml/100 g×min	1.7±0.08	0.82±0.04	1.3±0.16	1.1±0.09
pH	7.4±0.001	7.140±0.010	7.245±0.023	7.248±0.015
Concentration of buffer bases, mmol/liter	43.2±1.4	21.3±0.8	28.2±0.7	32.6±1.3
Concentration of bicarbonates, mmol/liter	20.5±0.9	6.0±0.5	4.5±0.3	9.3±1.2
Deficiency of buffer bases, mmol/liter	−3.90±0.39	−20.4±0.10	−19.1±0.70	−15.1±1.21
CO_2, mm Hg	33.70±0.87	18.00±1.00	10.7±1.00	21.74±0.28
Concentration of lactic acid, μmol/liter	2.45±0.13	4.61±0.14	3.85±0.27	3.40±0.31
Lipid peroxidation products, mmol/liter	1.14±0.05	2.41±0.12	2.13±0.28	2.45±0.51

of anxiety or aggressiveness on the part of the animals. The animals were then placed in a box divided into sections. Simultaneously, four rats were subjected to single-pulse electrostimulation at 5-10 minute intervals. The amplitude of the current of the pulses was 1.5-2 mA, and the duration of each pulse was 1.5 msec. As a result we were able to achieve a stable synchronization of stimulation and respiration, and the animals showed no signs of anxiety. The frequency of stimulation was 200 pulses/min. After the animals had spent time in the vivarium (experimental series II), had undergone adaptation to repeated sessions of electrostimulation (experimental series III), and had undergone adaptation to stress, they were subjected to acute hypoxic hypoxia by being made to inhale a gaseous mixture containing 6.5-7% oxygen for a period of 30 minutes. For this experiment tracheotomy was performed on the rats (anesthesized with 50 mg/kg chloralose and 500 mg/kg urethane, introduced intraperitoneally), and the common carotid artery and the opening of the venae cavae were catheterized. The parameters of external respiration and pulmonary gas exchange in the animals in all the series of experiments were determined with the aid of an automated device which we had developed for these purposes [4]. The gases and the acid-base equilibrium of the blood of the animals were analyzed with a microelectrode (Radiometer, Denmark). The ratio of the amount of oxygen carried by the blood to the oxygen demand was assessed according to the correlation between the supply and uptake (CSU) of oxygen. This correlation is numerically equal to the quotient resulting from dividing the rate of supply of oxygen carried by the arterial blood by the uptake of oxygen by the tissues. The degree of tissue hypoxia was assessed according to the concentration of lactic acid in the blood and tissues. This concentration was determined by the enzymatic method with the aid of standard kits of reagents (№. 256773, Boehringer, Germany). Lipid peroxidation was estimated by measuring the concentration of its products which entered into the reaction with thiobarbituric acid in the blood [9] and in the tissues [8]. The measurements were carried out with a spectrophotometer of the type F-4000 (Hitachi, Japan), and tetramethoxypropane (Sigma, USA) was used as the standard. The activation of lipolysis was assessed on the basis of the accumulation of free fatty acids (FFA) in the blood and organs [5]. Data on respiration and circulation in the animals in a state of sublethal hypoxia following adaptation to stress were taken from a work published earlier [1]. these data are given in Table 1 for purposes of comparison with the experimental results obtained with electrostimulation. In the adaptation to stress the animals lay on their backs in a fixed position for 30 minutes a day every other day for 12 days [1].

RESULTS

On the basis of the data obtained in the experiments (series I) we selected the parameters of the electrical

current to be used in electrostimulation in subsequent experiments. We also measured the current that was passed through the soft tissues of the head and brain. During electrostimulation we measured the density of the current passing through the skin of the head and the brain tissue, using platinum electrodes 1 mm in diameter. The measurements were made at 5 dorsal points lying in a straight line between the electrodes. The points were at an equal distance from one another. Two of them were at the base of each ear, and one was on the medial line.

The measurements showed that during electrostimulation the density of the current in the skin and in the subcutaneous layer near the electrodes was about 1 mA/cm^2 on the average. It decreased rapidly to 0.143 mA/cm^2 on the medial line as one moved farther away from the dorsal points on the medial line. The maximal and minimal values of the current density for the cortex and subcortical layer was 0.386 and 0.057 mA/cm^2, respectively. Approximately the same current density was registered in the sections that were symmetrical with respect to the medial line. These data indicate that electrostimulation as conducted in the present investigation was radically different from acupuncture in that it exerted an effect on both the soft tissues of the head and the brain. When electrostimulation was carried out under the conditions mentioned above, it led to hyperventilation owing to the high frequency of the current. In the last third of each respiratory cycle, when the acceleration of the gas flow had reached a second maximum, a new inhalation began when an electrical pulse was passed through the head. In Fig. 1, *a* crosshatching shows the time interval in which the amplitude of the pulse necessary for bringing about such premature inhalation was smaller than the pain threshold, which under our experimental conditions was about 3 mA. Thus, by changing the frequency of the pulses we can increase within certain limits (almost by 50%) the frequency of respiration. As can be seen from Fig. 1, *b*, there were several zones of synchronization. As the frequency of the pulses increased, breathing became more even. However, when the frequency of the stimulating pulses was increased to 400 per minute and higher, the animals that were in a waking state began to show signs of pain. Furthermore, as can be seen from Fig. 1, when the frequency of the pulses was more than 300 per minute (5 Hz), the synchronization was unstable since it could be attained at two different values of respiration frequency. Thus, it appeared convenient to select a frequency of electrical pulses in the range of 210 to 300 pulses/min (3.5-5 Hz); in three to four sessions of electrostimulation there was practically no change in the respiratory volume, oxygen uptake, or

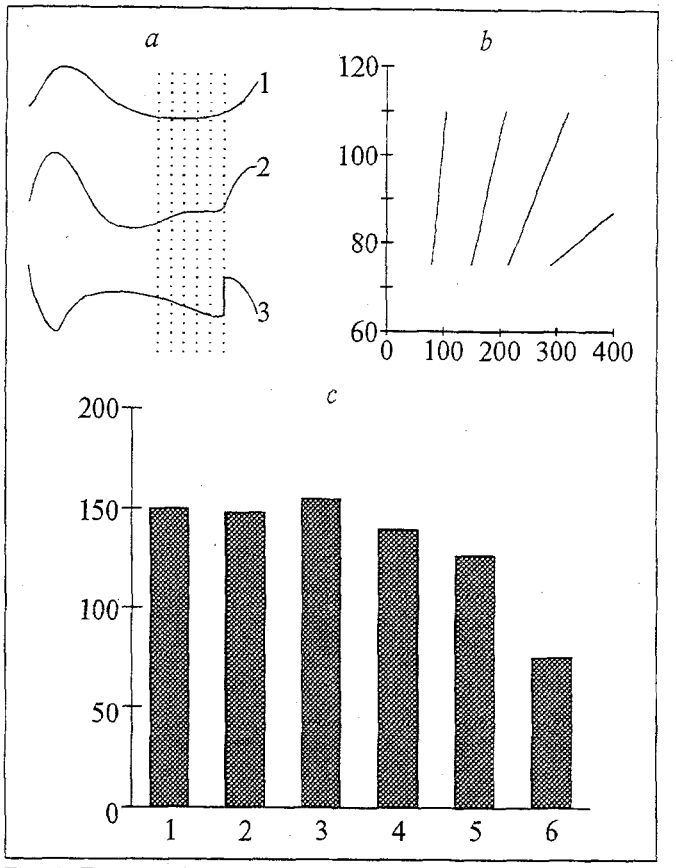

Fig. 1. Effect of electrostimulation on external respiration, blood flow, and gas exchange in rats under anesthesia. *a*) typical plots describing the change in lung volume (1) and in the rate (2) and acceleration (3) of gas flow in the respiratory airways (the zone of stable synchronization of breathing is crosshatched); *b*) frequency of respiration as a function of frequency of stimulating pulses; *c*) effect of electrostimulation on frequency of respiration (1), minute volume of respiration (2), circulation (3), alveolar ventilation (4), partial pressure of O_2 (5), and partial pressure of CO_2 (6) in the alveolar air in animals under anesthesia (the values for the parameters before electrostimulation are taken as 100%).

the discharge of carbon dioxide. The increase in the frequency of respiration during electrostimulation, therefore, led to an increase in the minute volume of pulmonary and alveolar ventilation and to the development of hyperventilation as regards oxygen uptake and the discharge of carbon dioxide (Fig. 1, *c*). Hyperventilation was indicated by an increase in the partial pressure of oxygen and a drop in the partial pressure of carbon dioxide in the alveolar air. Furthermore, electrostimulation led to an increase in blood flow. During such electrostimulation no activation of anaerobic glycolysis or lipid peroxidation was observed; i.e., tissue hypoxia did not develop. These results show that the type of electrostimulation being investigated can give rise to reactions from the oxygen-transporting systems that are similar to the compensatory shifts usually manifested under conditions of mild hypoxia [3] without, however, hypoxia

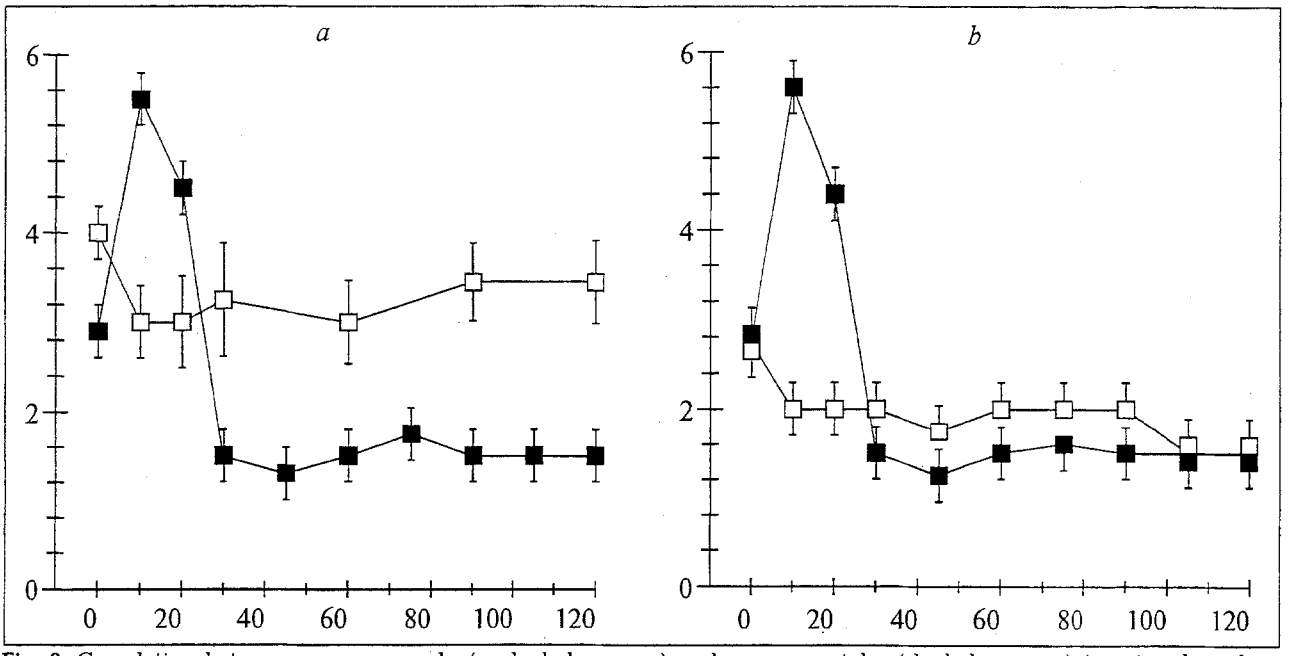

Fig. 2. Correlation between oxygen supply (unshaded squares) and oxygen uptake (shaded squares) in rats adapted to electrostimulation (*a*) and to repeated stress (*b*).

itself developing in the animals. Thus, the repeated electrostimulation procedure employed by us can be regarded as a kind of training of external respiration, but it cannot bring about adaptation to hypoxia in the strict sense, as the stimulus necessary for such a process was absent.

Our experiments showed that preliminary electrostimulation lowered the mortality of the animals more than twofold. In contrast, adaptation to stress reduced mortality 6.6 times [1]. Below is a detailed comparison of the effect of adaptation to electrostimulation and the effect of adaptation to stress on respiration, the circulation, and the acid-base equilibrium.

At least three conclusions can be drawn from the data in Table 1.

1. Preliminary adaptation to electrostimulation had a slight protective effect on external respiration. In acute hypoxia the increased frequency of breathing reduced the volume of air passing through the lungs in the respiratory cycle, and such an important parameter as the minute volume of respiration was the same as in animals not subjected to adaptation. On the other hand, adaptation to stress optimized external respiration in the case of acute hypoxia: the frequency decreased from 100 to 65 cycles; the volume of the respiratory cycle decreased 2.5 times as compared with the hypoxic control experiment and 2 times as compared with adaptation to electrostimulation; the minute respiratory volume increased by 40% as compared with the control experiment and by nearly as much as compared with adaptation to electrostimulation. To sum up, the partial pressure of O_2 in the arterial blood was 53% higher than in the hypoxic control experiment and 20% higher than that following electrostimulation. This was the result of economical yet effective external respiration.

2. Preliminary adaptation to electrostimulation considerably improved the circulation. However, the overall parameter, the transport of oxygen to the tissues, increased less than in the case of adaptation to stress. This was due to the low efficiency of external respiration.

Indeed, the shunt of pulmonary circulation - the passage of blood from the pulmonary artery directly to the veins - increased in the hypoxic control experiment from 6.2 to 44,5% (or 7 times). In animals adapted to electrostimulation the corresponding figure was even lower than the control values. At the same time, during adaptation to stress it increased 3.5 times. Accordingly, the minute volume of circulation after adaptation to electrostimulation increased more than 2.6 times, while after adaptation to stress it increased by only 25% as compared with the hypoxic control experiment. Thus, the amount of oxygen carried by the arterial blood, which decreased 3.6 times during hypoxia, increased more than 2.3 times after adaptation to electrostimulation as compared to that in the hypoxic control. On the other hand, after adaptation to stress it increased by only 40%. In other words, under conditions of acute hypoxia, adaptation to electrostimulation, by increasing the blood flow, maintained the transport of blood to the tissues on a higher level than did adaptation to stress.

3. In the case of adaptation to stress the amount of oxygen carried to the tissues was less than in the case of electrostimulation. Nevertheless, the amount

of oxygen consumed under conditions of acute hypoxia was considerably more in the case of adaptation to stress than in the case of electrostimulation. Thus, in hypoxia oxygen uptake (the decisive parameter) decreased from 1.7 to 0.82 ml/100 g×min in the control animals to 1.3 ml/100 g×min in the animals adapted to stress and to 0.98 ml/100 g×min in the animals adapted to electrostimulation. Thus, in acute hypoxia, despite the diminished amount of oxygen in the tissues, oxygen uptake increased by 60% in the animals adapted to stress and just by 20% in animals adapted to electrostimulation.

Figure 2, *a* shows the CSU of oxygen in the control animals and in animals adapted to electrostimulation under conditions of hypoxia. In the animals adapted to electrostimulation the CSU of oxygen increased nearly twofold, and the oxygen supply increased more than the oxygen uptake, with part of the oxygen being unused. It can be seen from Fig. 2, *b* that the CSU of oxygen changed little during adaptation to stress, and consequently the oxygen supplied was in the main consumed. These data indicate that the arterial-venous difference in pO_2 in animals adapted to stress was 2.5 times greater than in animals adapted to electrostimulation. Thus, by increasing the blood flow, electrostimulation ensured a greater increase in the amount of oxygen carried to the tissues than did adaptation to stress. However, by reducing the respiratory volume, adaptation to stress ensured a higher pO_2 in the arterial blood. Thus, in acute hypoxia oxygen uptake by the tissues was three times greater after adaptation to stress than after adaptation to electrostimulation. Correspondingly, mortality in acute hypoxia was three times lower after adaptation to stress than after adaptation to electrostimulation.

The data in Table 1 show that sublethal hypoxia caused a decrease in the pH of the blood, a drop in the concentration of the buffer bases and bicarbonate, an especially marked hyperventilation, an increase in the concentration of lactic acid in the blood, and a manifold increase in the concentration of malonic dialdehyde (MDA). Adaptation to stress, as shown above, substantially limited all these shifts except for bicarbonate deficiency and hyperventilation. The magnitude of these shifts in animals that underwent preliminary adaptation to stress was much greater than in the control hypoxia experiment. This was obviously due to the greater pulmonary ventilation, i.e., to an increase in pulmonary respiratory volume and an increase in CO_2 discharge from the organism (Table 1). As a result the concentration of lactic acid increased less, and the pH increased more, than in the control hypoxia experiment. Furthermore, adaptation to stress had no effect on the MDA level in the blood, but decreased the concentration of FFA in the blood 3.5 times. In other words, it forestalled the hypoxic activity of lipolysis. Adaptation to electrostimulation, like adaptation to stress, limited the drop of the pH of the blood and the rise in the concentration of lactate in the blood. However, unlike adaptation to stress, it did not cause additional hyperventilation, and it limited the decrease in the concentration of the buffer bases and bicarbonate. It also had no effect on the concentration of MDA and FFA in the blood.

In the determination of the concentration of lactic acid, MDA, and FFA in the internal organs it was found that electrostimulation hindered the accumulation of lactic acid in the internal organs to a lesser extent than did adaptation to stress. However, it decreased the concentration of lactate twofold in the lungs and in the skeletal muscles. Also, under the influence of electrostimulation the concentration of MDA decreased, but the concentration of FFA did not, as compared with the hypoxic control. Thus, the antihypoxic effect of adaptation to electrostimulation, which in fact led to an enforced 15-minute tachypnea, consists in the elimination of the pulmonary shunt, an increase in the general blood flow, and an increase in the amount of oxygen carried to the tissues. As a result, oxygen uptake by the tissues increases, if only moderately (by 20%). These important shifts are not accompanied by hyperventilation and are therefore linked to a limit on the deficiency of buffer bases and bicarbonate. This kind of adaptation to electrostimulation reduces mortality more than twofold in the case of sublethal hypoxia.

In the adaptation to stress the antihypoxic effect is of a different nature. It consists mainly in the effective and steady promotion of breathing for a relative lowering of the level of minute blood flow and a decrease in the amount of blood carried to the tissues, but on the other hand an increased pressure of O_2 in the arterial blood and a considerably increased (by 60%) oxygen uptake by the tissues under conditions of sublethal hypoxia. This strategy of adaptation is more effective as it raises the animals' resistance to sublethal hypoxia 6.5 times. The mechanism of this effect has been discussed earlier. In the present work it is important to note the practical aspect: adaptation to immobilized stress is not applicable to humans; on the other hand, electrostimulation, which caused some drowsiness in the animals, had an antihypoxic effect, and with the right set of parameters of the electrical current it can be used for boosting resistance to hypoxia in man.

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Correction of Disturbances of Cardiac Electrical Stability in Postinfarction Cardiosclerosis with a Polyunsaturated Fatty Acid-Enriched Diet

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It has been shown previously that polyunsaturated fatty acids (PUFA) added to the rat diet as eiconol preparation with a high content of docosahexaenoic and eicosapentaenoic acids reduce mortality for acute myocardial infarction sevenfold. This result has been achieved mainly due to suppression of ischemic and reperfusion arrhythmias and, particular, heart fibrillation [1,7]. These facts agree with the results of epidemiological and clinical investigations that have shown fish oil consumption to reduce mortality in myocardial infarction [3,8]. The influence of a PUFA-enriched diet on the contractility and electrical stability of the heart during postinfarction cardiosclerosis (PIC) has not yet been investigated. Meanwhile, millions of people surviving infarction suffer from PIC, posing an urgent problem in clinical cardiology. A considerable proportion of these people die from severe arrhythmia culminating in fibrillation [2,6].

The aim of the present study was to assess the effect of a PUFA-enriched diet on spontaneous arrhythmia and the main parameters of the electrical stability of the heart in animals with PIC.

MATERIALS AND METHODS

Experiments were carried out on Wistar male rats weighing 300±10 g. The animals were divided into four groups: group 1 comprised intact animals (control); group 2 consisted of animals receiving an eiconol-containing diet for 50 days; group 3 comprised animals with a 30-day myocardial infarction (i.e., with PIC) receiving the standard diet; group 4 comprised animals in which myocardial infarction was created after they had received an eiconol-containing diet during 15-23 days, after which they were kept on the same diet for another 30 days. Myocardial infarction was reproduced after Selye. Eiconol

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