Development of Resistance of an Organism under Various Conditions of Hypoxic Preconditioning: Role of the Hypoxic Period and Reoxygenation

L. D. Lukyanova, E. L. Germanova, and R. A. Kopaladze

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Single exposure to moderate (10% O_2) hypobaric, normobaric, and intermittent hypoxia is followed by a preconditioning response of the organism. The mechanisms for immediate adaptation are activated during the hypoxic period. Intermittent reoxygenation not only delays, but even suppresses this process. However, periods of oxygenation during the course of hypoxic training reduce the effect of hypoxia and prevent the possibility for "overdosage" of the adverse stimulus. Hence, they have a regulatory or normalizing role under these conditions. Our results indicate that hypoxitherapy in intermittent hypoxia mode provides optimum conditions for long-term adaptation.

Key Words: *hypobaric, normobaric, and intermittent hypoxia; reoxygenation; preconditioning; resistance*

Even one episode of non-damaging hypoxic or ischemic exposure increases the resistance to subsequent severe hypoxia (preconditioning). This effect is observed under *in vivo* and *in vitro* conditions. The protective effect develops immediately after short-term moderate hypoxia (2-5 min) and persists for several days [9-10]. These changes are accompanied by a decrease in postischemic release of creatine kinase, prevention of cytochrome *c* loss (relative preservation of the outer membrane) and apoptosis activation. The respiratory rate on NADH-dependent substrates decreases, but returns to normal in the presence of succinate, tetramethyl-*p*-phenylenediamine, and ascorbate. These changes illustrate inhibition of electron transport function of mitochondrial enzyme complex I, but not of complexes II, III, and IV [1-8]. The respiratory control remains unchanged under these conditions. Therefore, the synthesis of ATP is not impaired. Preconditioning has no effect on $\Delta\psi$. Hence, the proton permeability and permeability of the inner mitochondrial membrane do not change during preconditioning.

Various types of hypoxic preconditioning are accompanied by an increase in the nonspecific resistance of an organism. This procedure is extensively used in preventive and therapeutic medicine (hypoxitherapy). The method of intermittent normobaric hypoxia (INH) is of particular importance in this respect. Over the last 15-20 years, this simple method was used instead of altitude chamber training under conditions of hypobaric hypoxia. It was hypothesized that the therapeutic effect of INH exceeds that of other types and regimens of hypoxic training. However, this notion is not supported by the results of experimental studies. No comparative studies were performed to evaluate the effect of various types of hypoxic training on the development of resistance to hypoxia.

Here we compared the efficacy of various types of hypoxia in the development of adaptation.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. Russia. ***Address for correspondence:*** ldluk@mail.ru. L. D. Lukyanova

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MATERIALS AND METHODS

Experiments were performed on male albino rats with different initial resistance to hypoxia. One month before the start of the study, experimental animals were tested for the resistance to acute hypobaric hypoxia. They were elevated to a critical height of 11,500 m in an altitude chamber. We measured the time to loss of posture and locomotor activity (TLP_1), which usually occurred at a subcritical height of 10,000-10,500 m ("lying on the side" posture). The lifetime of rats (LT_1) was evaluated from the moment of reaching the critical height to the second agonal breath. The first parameter reflects the overall response of the organism to hypoxia at subcritical concentrations of oxygen (*i.e.*, locomotor activity and respiration). The second parameter characterizes the ability of animals to mobilize their protective mechanisms for survival under life-incompatible extreme conditions.

Functional and metabolic parameters of rats returned to normal 1 month after studying the resistance to acute hypobaric hypoxia. The animals were randomized into two groups of specimens with low (group 1) and high resistance to hypoxia (group 2).

We studied the effect of single exposure to various types of preconditioning with moderate hypoxia (10% O_2) on general resistance of the organism (TLP and LT). Hypoxic training was conducted under the following conditions: (1) single exposure of animals to hypobaric hypoxia (reduced barometric pressure) in an altitude chamber at a height of 5000 m and 10% O_2 for 60 min; (2) single exposure of animals to normobaric hypoxia (atmospheric barometric pressure) in a chamber at 10% O_2 for 60 min; and (3) single exposure of animals to INH (INH-20, total duration 60 min). During 6 cycles of INH, short-term periods of passive breathing of a hypoxic gas mixture at constant pressure (10% O_2, 5 min) alternated with breathing of atmospheric air (3 min).

The effect of preconditioning on immediate adaptation in low-resistance and high-resistance rats was evaluated in the following periods: immediately after treatment; 30 and 60 min after treatment; 2, 4, and 24 h after treatment; and 2 and 3 days after treatment. During these periods, all rats were repeatedly "elevated" to a critical height in an altitude chamber. TLP_2 and LT_2 were measured. The TLP_2/TLP_1 and LT_2/LT_1 ratios were calculated (in percent; adaptation coefficients).

The development of long-term resistance to hypoxia was estimated after the course of hypoxic training under various conditions. The resistance was evaluated from TLP and LT on day 1 after daily training for 7, 15, and 21 days.

RESULTS

Preconditioning with 3 types of hypoxia was followed by an increase in the resistance of animals to acute hypobaric hypoxia.

A significant increase in the resistance of animals was observed immediately after hypoxia. LT of rats with low resistance to hypoxia increased by 650, 550, and 375% after 60-min training under conditions of hypobaric hypoxia, normobaric

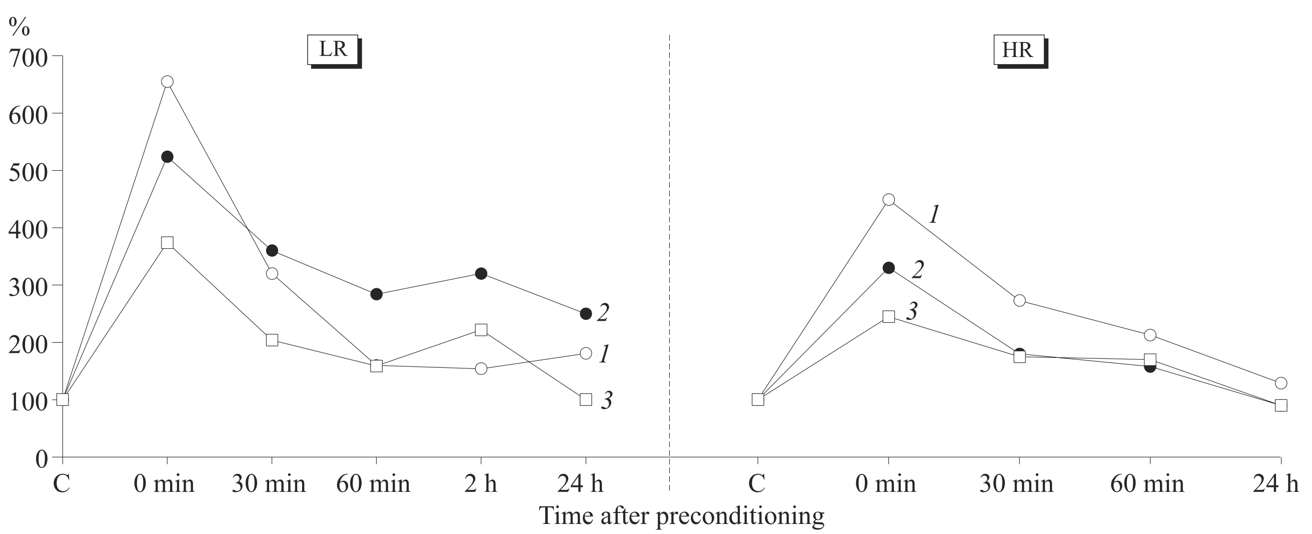

Fig. 1. Effect of single 1-h exposure to hypobaric hypoxia, normobaric hypoxia, and INH-20 on the resistance of low resistant (LR, *a*) and highly resistant rats (HR, *b*) during the posthypoxic period (LT at a critical height of 11,500 m). Hypobaric hypoxia (*1*), normobaric hypoxia (*2*), and INH-20 (*3*).

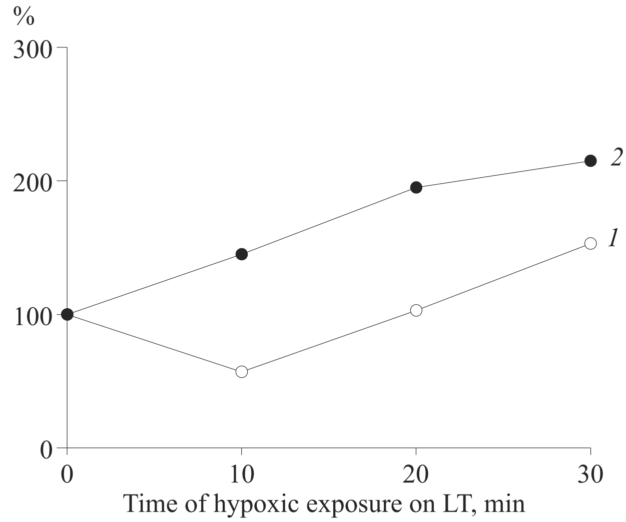

Fig. 2. Dependence of LT of low resistant rats on the time of exposure to the hypoxic stimulus during training with INH-20 (*1*) and continuous normobaric hypoxia (*2*).

hypoxia, and INH (Fig. 1). The preconditioning-induced resistance to acute hypoxia decreased rapidly during reoxygenation and was minmum after 60 min. However, the resistance of rats to hypoxia during this period remained above the baseline value (by 1.5-3 times, Fig. 1). No significant differences in the results were found after exposure to various types of hypoxia.

Various types of hypoxia had similar effect on the resistance of highly resistant rats (time and amplitude of changes). This conclusion was derived from studying the parameter of LT. However, quantitative changes in the resistance of highly resistant animals were less pronounced compared to low resistant specimens (Fig. 1).

Our results indicate that preconditioning with any type of hypoxic training contributes to the resistance of an organism to acute hypoxia. The effect was most significant after exposure to continuous hypobaric and normobaric hypoxia. These data suggest that the period of reoxygenation, a component of INH cycle, reduces the efficacy of resistance development.

Activation of mechanisms for resistance development is exlusively related to the hypoxic period of training. The protective antihypoxic effect was most pronounced immediately after preconditioning, but decreased rapidly in the follow-up period.

Moreover, the protective effect of preconditioning depends strongly on the duration of hypoxic training and shortening of this period during reoxygenation (Fig. 2).

The resistance of an organism (LT) was shown to increase 10 and 30 min after continuous normobaric hypoxia (by 1.5 and 2.2 times, respectively; Fig. 2). LT decreased by 40% over the first 10 min of INH-20, but progressively increased in the follow-up period. Thirty minutes after hypoxic exposure, LT of these animals was much lower compared to rats of the normobaric hypoxia group and control specimens (by 1.5 times).

Changes in the resistance of animals after single training with INH-20 were less pronounced compared to those observed during continuous hypoxic exposure. It can be concluded that intermittent oxygenation not only delays, but even suppresses this process.

During the course of hypoxic training (20 daily training under conditions similar to those in single exposure), the development of long-term adaptation depended on the type of hypoxic exposure. Single exposure to continuous hypoxic training was more effective than INH-20 in inducing the development of immediate adaptation. However, these differences were not observed after course training. For example, the increase in the resistance of low resistant rats was revealed only after the first three sessions of training with hypobaric hypoxia (2-fold increase in LT, Fig. 3). The response to hypoxic exposure became less pronounced in the follow-up period of training. TLP remained unchanged under these conditions. A slight increase in TLP was found only after 15 sessions of training. Therefore, the conditions of training with normobaric hypoxia were not optimal. This treatment did not provide the development of long-term adaptation. It was probably related to “overdosage” of a continuous hypoxic stimulus and subsequent depletion of compensatory adaptive reserves.

TABLE 1. Effect of the Course of INH-20 and INH-30 on LT of Low Resistant Rats

Regimen of treatment	Number of daily training sessions				
	0	1	8	15	21
INH-20	100	185	275	550	190
INH-30	100	265	385	270	146

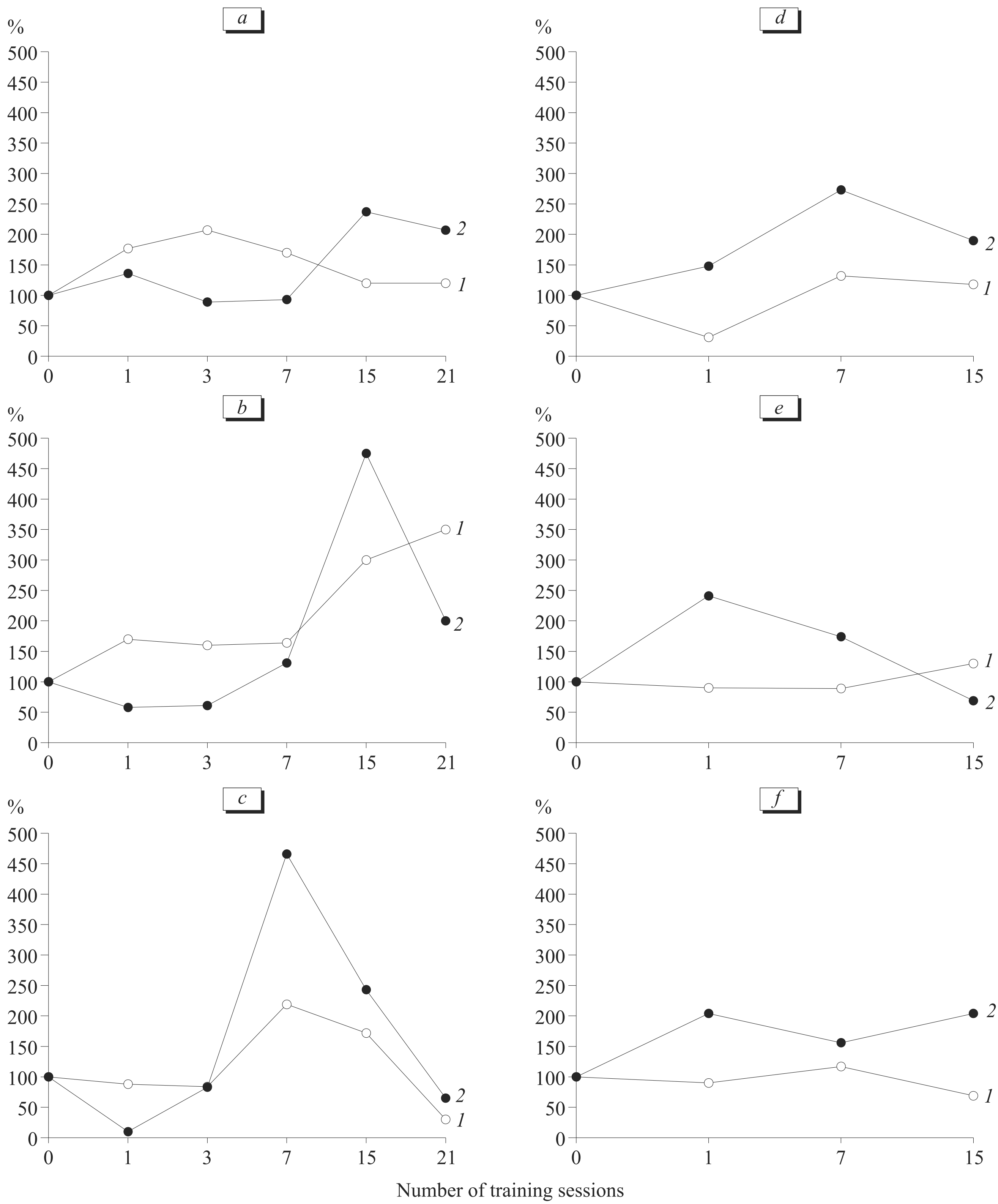

Fig. 3. Effect of the course of various types of hypoxic training on resistance development in low resistant and highly resistant rats during the posthypoxic period. Hypobaric hypoxia in low resistant rats (LR, *a*); INH-20 in LR rats (*b*); continuous normobaric hypoxia in LR rats (*c*); hypobaric hypoxia in highly resistant rats (HR, *d*); INH-20 in HR rats (*e*); continuous normobaric hypoxia in HR rats (*f*). LT (*1*) and TLP (*2*).

More significant changes were observed after the course of normobaric hypoxia. However, this treatment produced only a short-term increase of the resistance in low resistant rats. These changes were preceded by a decrease in the resistance to hypoxia (Fig. 3).

The development of long-term resistance in low resistant animals was most pronounced after INH-20. The course of hypoxitherapy (up to 7-15 sessions of training) was accompanied by an increase in LT (by 3-3.5 times) and TLP (by 4-4.5 times; Fig. 3). We conclude that the development of adaptation under these conditions is related to a variety of physiological functions.

Periods of oxygenation in hypoxitherapy probably have a regulatory or normalizing role. They reduce an adverse effect of hypoxia and prevent the possibility for "overdosage" of hypoxia. During repeated exposure to hypoxia under conditions of INH-20, oxygenation periods provide optimum conditions for long-term adaptation.

It should be emphasized that the degree of oxygenation during the inter-hypoxic period plays an important role in adaptation. The generation of reactive oxygen species during reoxygenation probably has a signal function, which is required for the activation of immediate and long-term mechanisms of adaptation. Hence, the increase in oxygen concentration in the inspired air during the inter-hypoxic period of INH should contribute to acceleration of this process.

However, this assumption was not supported by the results of experiments with INH-20 and INH-30. The resistance of low resistant animals increased similarly over the first seven trainings under these conditions. During the follow-up period, the resistance increased sharply in the INH-20 group, but remained unchanged in the INH-30 group (Table 1). We conclude that the increase in oxygen concentration in the inspired air (up to 30%) does not improve, but even suppresses the development of long-term adaptation.

The development of resistance was shown to differ in highly resistant and low resistant animals. None of the types of hypoxitherapy (including INH-20) induced an increase in the resistance of highly resistant rats to oxygen at the critical concentration. LT of these animals did not increase under these conditions. Moreover, normobaric hypoxia was accompanied by a decrease in LT of rats. However, highly resistant animals were adapted to hypoxia at the subcritical concentration of oxygen. This conclusion was derived from the increase in TLP. TLP increased most significantly after 7 sessions of training. At the same time, these changes in highly resistant animals were less pronounced than in low resistant specimens (Fig. 3).

The response of an organism to acute hypoxia was reduced after 15 or 7 sessions, which did not depend on the type of hypoxic training. The observed changes reflect complete development of adaptation under these conditions, including INH-20 (Fig. 3). Therefore, the course of hypoxitherapy should not exceed 12-15 sessions of training.

Studying the effect of various types of hypoxitherapy on the resistance of the organism showed that the mechanisms for adaptation to hypoxia are activated during exposure to the hypoxic stimulus, but become suppressed in the period of reoxygenation. The course of INH-20 has several advantages over continuous hypoxic exposure. The period of oxygenation reduces an adverse effect of the hypoxic stimulus and prevents the development of overdosage-induced complications. Hence, they have a regulatory or normalizing role. The course of INH-20 (up to 15 sessions of training) provides optimum conditions for long-term adaptation.

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