Effect of Acute Hypoxia on Resistance to Hypoxia in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 6, pp. 625-628, June, 1999
Original article submitted May 21, 1998

The resistance of rats to hypoxia was measured by survival time after exposure to high-altitude (11.5 km) hypoxia. The first exposure to acute hypoxia caused phasic changes in the survival time: short-term in high-resistant rats (about 24 h) and long-term in moderate- and low-resistant rats (38-39 days) starting from 1 h and 6-7 days after the first exposure, respectively. Adaptive reactions were more pronounced in low- and moderate-resistant rats, while disadaptation was typical of high-resistant animals. In all rats, the adaptive effect dominated until days 22-23. Throughout the testing, the initial type of resistance was retained in 79% of high-resistant rats, in 41% of low-resistant and in 33% of moderate-resistant rats, i. e., the initially homogenous groups formed after the first exposure in accordance with the type of resistance became mixed, which reduced the intergroup differences.

Key Words: *acute hypoxia; resistance*

Body resistance to hypoxia depends on a variety of factors: daytime [3,12,16], season [3,13], feeding [16], and others. In animals, the resistance to acute hypoxia was tested by exposure to acute hypobaric hypoxia which can change their initial resistance [3,4, 13]. The purpose of this study was to assess the effects of short-term hypoxic exposure on the resistance to acute hypoxia in rats.

MATERIALS AND METHODS

The resistance to acute hypoxia was tested in male Wistar rats. They were exposed to acute hypoxia by "lifting" in a pressure chamber to an altitude of 11.5 km above the sea level for 60 sec. The experiments were carried out during daytime (13.00-21.00) in autumn. The resistance to acute hypoxia was measured by the survival time under hypoxic conditions, i. e., time after "ascent" to reversible respiratory arrest, after which the animal was "descended" [3,4]. The first exposure determined the initial resistance to acute hypoxia and served as a short-term conditioning exposure. The second test was performed after various time intervals (Fig. 1).

From the log-normal distribution of the survival time (n=374) during the first exposure we determined the ranges for low, moderate and high resistance to hypoxia (LR, MR and HR, respectively) for every hour of daytime [12] and used them for the assessment of resistance during the first and second tests. Since the resistance to hypoxia depends on the daytime [12], a single group of resistance (n=191) was formed by rats that were tested in 3 intervals (13.00-15.00; 16.00-18.00, 19.00-21.00) during the first exposure. This daily dynamics was taken into considerations during the second exposure. Body weight increased from 150-180 g to 250-280 g by the end of testing (33-39 days). The data were statistically analyzed using "Statgraphics" and "Statistica" software. Because of asymmetric distribution of survival periods, the median was used as the center of distribution [5]. Nonparametric one-way analysis of variance, median criterion, and the Kruskal—Wallis test were applied to analyze the effects of short-term hypoxic exposure on the survival time. The Mann—Withney U-test was used for paired comparison. The χ^2 test was applied to compare the size of groups with different resistance to hypoxia. In all the tests the null hypotheses were rejected at the significance level of α=0.05.

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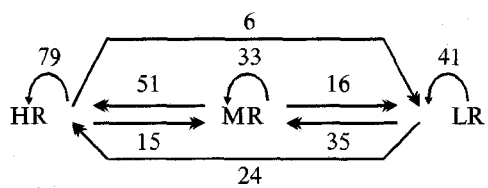
0007-4888/99/0006-0567$22.00

RESULTS

Short-term hypoxic exposure changed the survival time in different groups of rats with different initial resistance to hypoxia: in the HR group, this effect was observed from 13.00 to 21.00 (p<0.05-0.01), in LR and MR rats it was manifested in the 16.00-18.00 and 19.00-21.00 intervals (survival time during the second test was 1.2-1.8 times longer than during the first test, p<0.05-0.01). The changes caused by short-term exposure were characterized by different dynamics and direction: in HR rats, they manifested themselves early (within 24 h), in LR rats they occurred after 6-7 days, and in MR rats they were observed both in the early and late periods. The survival time of HR rats increased 1 h after the first exposure (Fig. 1, *a*), returned to the initial level after 6 h, dropped below it after 24 h, and starting from days 6-7 did not differ from the initial level. In LR and MR rats, the survival time showed several peaks with the initial values between them: in the LR group these peaks were reached on the 6-7th, 22-26th, and 38-39th days; in the MR rats - on the 1st, 6-7th, and 38-39th days. The peak values 1.1-2.4-fold surpassed the initial ones (p<0.05-0.01). Two phases were revealed in the dynamics of survival time in HR and MR groups (Fig. 1, *b*): a gradual decrease by the end of the 1st day in RH rats and by the 25-26th days in MR rats (as a result, the peak values after 1 h and the minimal values recorded in corresponding periods differed by 3.8 times in the HR group and 2.4 times in the MR group, p<0.01), followed by its increase after 6-7 and 38-39 days, respectively. The group of LR rats showed no difference in the survival time in these time intervals. It should be noted that in the majority of points tested, the groups became heterogenous with respect to the resistance to hypoxia because of changes in the individual survival time. The most stable group was HR rats: 79% of them were classified to the same group during the experiment (100% after 1 h). Only 41 and 33% of LR and MR rats, respectively, showed the same type of resistance during the second test (p<0.01). The changes in the type of resistance during examination period expressed in percents of the initial sizes of the groups can be illustrated by the following scheme:



Thus, the type of resistance during the second test changed in almost 50% rats. Within the interval from 1 h to 22-23 days after the first exposure, the number of HR rats increased 2.4 times with a simultaneous decrease in the number of LR and MR rats (1.9 and 1.4 times, respectively, p<0.05-0.01). The groups returned to their initial sizes by the end of the test-period. When calculating the number of rats with 25% or higher deviations of the survival times from the initial values (data not presented) we found that although survival time in all groups increased or decreased, the increase was more often in the LR and MR groups, while its decrease was more characteristic of the HR group (2-3.2 times, p<0.01). As a result, the initially homogenous groups showed significant differences between the survival periods at different times after the first test: 7.3-, 5.4-, and 3.6-fold differences in the HR, LR and MR groups, respectively (p<0.01).

Individual variations in the survival time during the second test modified the initial difference between the groups (Fig. 1, *a*). At some points, the initial relationships between the survival times in different groups (HR:MR:LR=3.6:1.5:1 (p<0.01)) disappeared. The most stable differences between the HR and LR groups disappeared after 1 and 38-39 days. The differences between all groups persisted for 1 h and appeared again 33-34 days after the first exposure.

Thus, short-term hypoxic exposure increases the resistance to hypoxia in rats. The increased resistance reflects potentiation of the mechanisms of immediate adaptation to hypoxia. It can be suggested that the first exposure to hypoxia acts as a stress factorm, which induces a stress response activating the mechanisms of immediate adaptation to hypoxia, i. e., cross-effects of adaptation to hypoxic and stress conditions can manifest themselves not only in long-term [10], but also in immediate adaptation. In fact, hypoxia is a stress-factor [1] activating the hypothalamo-pituitary-adrenal and sympathico-adrenal systems [1,9], which is confirmed by elevation of blood corticosterone 1.5 min after exposure to acute hypoxia [6]. A stress-syndrome has several phases, which determines phasic changes in the resistance to hypoxia. This dynamics corresponds to phasic changes in the catecholamine content in the adrenal medulla observed from the first hours of hypoxia [14] with massive mobilization of endogenous catecholamines [17]. The latter seems to occur also after short-term exposure to hypoxia, being accompanied by an increased fluorescence of myocardial adrenergic terminals not only in the early (6 h) but also in the late periods (3 weeks) after acute hypoxia [11]. In our study, the resistance to hypoxia in LR rats increased 6-7 day after the first exposure, i. e. in the late period. At the same time, in HR rats, the resistance to hypoxia increased as soon as 1 h after the first exposure and dropped below the initial value by the end of the first day. This difference is probably due to different dynamics of stress-response in animals

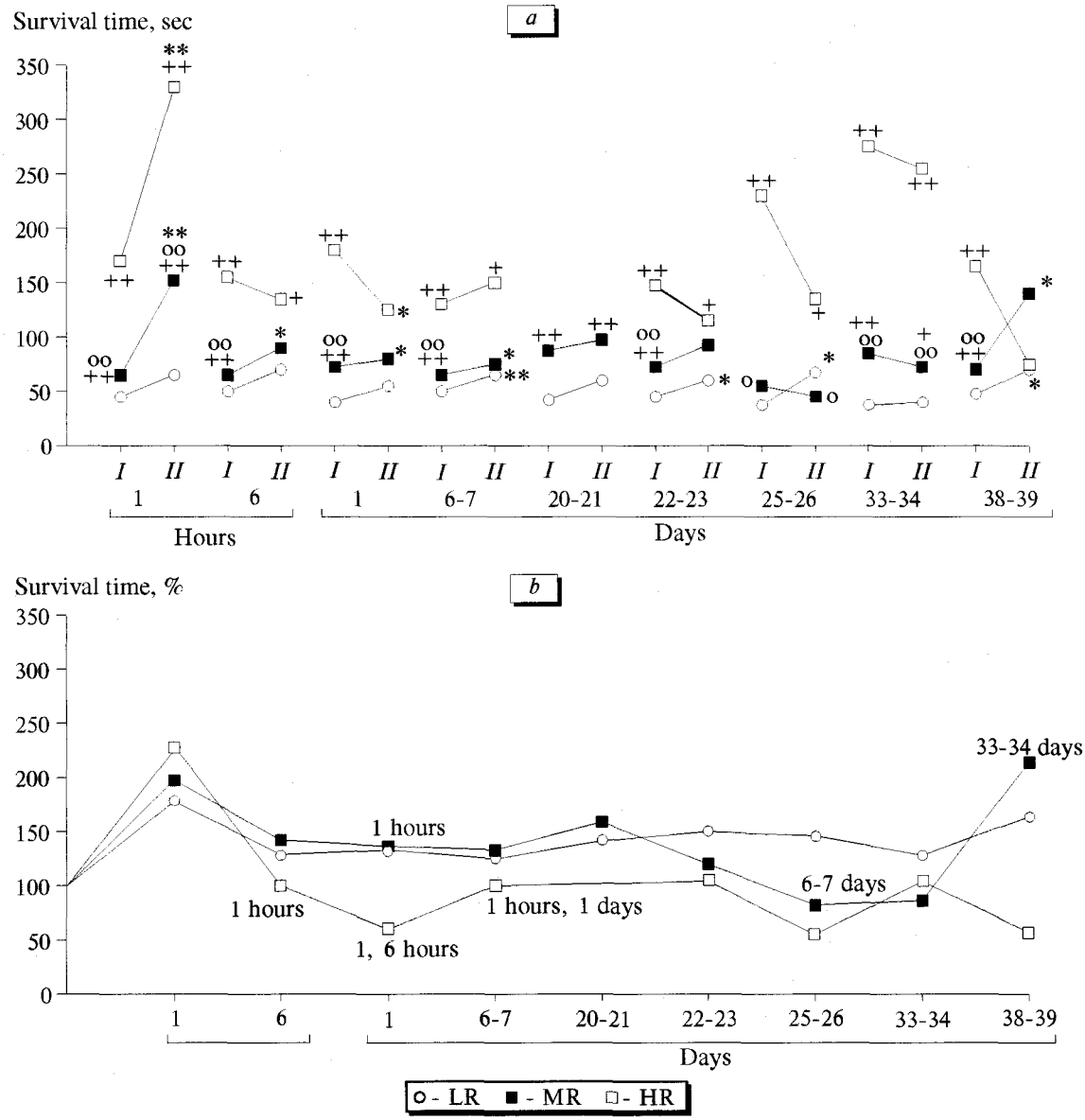


Fig. 1. Median values for survival time during the first and second hypoxic exposures (*a*) and relative changes of this index in the second test (*b*). *a*: *I*, *II*, the first and second tests, respectively; **p*<0.05, ***p*<0.01, compared with the first test; +*p*<0.05; ++*p*<0.01, compared with low-resistant rats; °*p*<0.05, °°*p*<0.01 compared with high-resistant rats. *b*: Figures above symbols indicate the time points (hours, days) when the differences were significant (*p*<0.05-0.01). The number of rats exposed to both tests: 4-13 in the groups of low and mediate-resistant rats; 5-7 in the high-resistant group.

with initially different resistance to hypoxia. In HR rats, the system of immediate adaptation can be more labile and readily exhausted. In LR rats, this system seems to be more inert but has greater reserve capacities. It can be suggested that LR rats have higher potential for the formation of a systemic structural trace of adaptation to hypoxia [9] than HR rats. This suggestion is supported by the data on increased resistance to hypoxia in LR rats after long-term (30-days) adaptation to hypoxia, which either reduced or did not affect the resistance to hypoxia in HR rats [13]. It is likely that even the animals referred to the same group of resistance have different adaptive capacities, due to which the hypoxic episode transforms the initially homogenous group into a mixed one eliminating the initial intergroup difference. It implies that the same resistance can be achieved by different stress on the immediate adaptation mechanisms. Since the rats of the same age and weight exhibited different changes in the resistance to hypoxia in the late period after short-term exposure, the age factor hardly affected its dynamics. Besides, by the first exposure the age of our rats exceeded 60 days, i. e. they had reached the adult level of resistance (until this age the resistance to hypoxia greatly varied) [7]. The key role in the immediate adaptation and in the formation of the sys-

temic structural trace is played by ATP resynthesis [9]. ATP resynthesis can be activated by increased glucose concentration in hippocampal neurons [15] and high succinate dehydrogenase activity, as well as by switching to the dicarbonic part of the Krebs cycle in the rat brain and liver (which is more pronounced in HR rats) [4], relatively high intensity of NAD-dependent oxidation in the brain [8,13], succinate oxidase pathway in the myocardium of HR rats [8], and other mechanisms underlying the immediate adaptation to hypoxia. In LR rats, hypoxia impairs the NAD-dependent oxidation in the respiratory chains of cerebral and myocardial cells [8]. Activation of this metabolic pathway in the brain [13] and myocardium [7] could serve as a mechanism of long-term adaptation in LR rats. Changes in the resistance to hypoxia caused by single hypoxic eposide or by adaptation do not contradict with the concept of genetic determination of the resistance to hypoxia [4]. What seems to be genetically predetermined is the capacity to change the resistance to hypoxia within certain limits, which is a feature with a broad reaction norm. This suggestion can be applied not only to the entire population of rats but also to HR, MR, and LR groups.

REFERENCES

1. N. A. Agadzhanyan and A. I. Elfimov, *Body Functions under Conditions of Hypoxia and Hypercapnia* [in Russian], Moscow (1986).
2. N. A. Agadzhanyan, L. V. Sorokin, E. P. Tambovtsev, and V. I. Torshin, *Byull. Eksp. Biol. Med.*, **120**, No. 9, 239-241 (1995).
3. N. A. Agadzhanyan, V. I. Torshin, and V. E. Starykh, *Ibid.*, **114**, No. 11, 523-525 (1992).
4. V. A. Berezovskii, K. A. Boiko, K. S. Klimenko, *et al.*, *Hypoxia and Individual Responsiveness*, Ed. V. A. Berezovski [in Russian], Kiev (1978).
5. Yu. N. Blagoveschenski, V. P. Sameonova, and E. A. Dmitriev, *Nonparametric Methods in Soil Research* [in Russian], Moscow (1987).
6. T. V. Goryacheva, A. M. Dudchenko, M. E. Spasskaya, *et al.*, *Byull. Eksp. Boil. Med.*, **116**, No. 7, 46-48 (1993).
7. A. A. Korneev, I. A. Komissarova, and Ya. R. Nartsissov, *Ibid.*, No. 9, 261-263
8. L. D. Luk'yanova, in: *Pharmacological Correction of Hypoxic Conditions* [in Russian], Moscow (1989), pp. 11-44.
9. F. Z. Meerson, *Adaptation, Stress and Prophylaxis* [in Russian], Moscow (1989).
10. F. Z. Meerson and M. G. Pshennikova, *Adaptation to Stress Conditions and Physical Load* [in Russian], Moscow (1988).
11. M. L. Khachatur'yan, *Morphological and Biochemical Changes in the Myocardium and Adrenal Catecholamines under Conditions of Norepinephrine Stress in Rats with Different Resistance to Hypoxia (Experimental Study)*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1993).
12. M. L. Khachaturyan, L. A. Panchenko, E. N. Zanina, *et al.*, *Byull. Eksp. Biol. Med.*, **127**, No. 3, 256-260 (1999).
13. G. N. Chernobaeva and L. D. Luk'yanova, *Pharmacological Correction of Hypoxic States* [in Russian], Moscow (1989), pp. 160-164.
14. Y. Hayashi, S. Miwa, K. Lee, *et al.*, *J. Neurochem.*, **54**, No. 4, 1115-1121 (1990).
15. I. S. Kass and P. Lipton, *J. Physiol.* (Lond.), **413**, 1-11 (1989).
16. T. Masukawa and Y. Tochino, *Jpn. J. Pharmacol.*, **61**, No. 3, 197-200 (1993).
17. C. E. Rose, J. A. Althaus, and D. L. Kaiser, *Am. J. Physiol.*, **245**, No. 6, H924-H929 (1983).