Changes in Hypoxia Resistance in Rats during Daytime

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The resistance to acute hypoxia in male Wistar rats was evaluated by the period of survival after exposure to high-altitude hypoxia (11.5 km above see level). The study was performed during daytime (13.00-21.00) in autumn. The fatal rat population was characterized by the log-normal distribution of survival periods. The rats with low and moderate resistance to hypoxia exhibited similar diurnal variations in it with gradual decrease by the end of daytime more pronounced in low-resistant rats. The rats with high resistance showed relatively constant resistance to hypoxia which decreased only at 21:00. All groups revealed a relatively stable resistance to hypoxia from 16:00 to 18:00. These variations in the resistance to hypoxia should be taken into consideration when planning experimental research.

Key Words: *resistance to hypoxia; diurnal rhythm*

Reportedly, rats can be classified as low-resistant (LR), moderate-resistant (MR), and high-resistant (HR), when their resistance to hypoxia is measured by the survival period (SP) under acute hypoxic conditions [1,3,4,8,10]. It has been proposed to calculate the mean SP value as an arithmetic mean for the total population and to determine the boundaries between MR and other groups as symmetrical points around the mean (for instance, ±30% of its value; this range is allowed to be voluntarily extended or narrowed [4]). The majority of studies are based on the assumption that SP is distributed normally. Then the population mean represents the center of distribution, and the boundaries between groups with different resistance to acute hypoxia (RAH) are determined empirically. Recently, an asymmetrical, log-normal type of SP distribution has been revealed. It has been proposed to calculate a geometric mean as its center and to assume that the number of animals with different RAH is equal. It has been shown that there can be significant differences (up to 30%) between SP values computed as an arithmetic and a geometric mean [1]. It is known that SP undergoes seasonal and circadian variations [2]. However, no information is available on circadian changes in SP of inbred rats and animals with different RAH.

Our aim was to reveal the type of distribution of SP values during daytime, to determine the range of SP values for rats with different RAH, and to investigate variations of RAH during daytime.

MATERIALS AND METHODS

Male Wistar rats (n=374) weighing 150-180 g were used. Experiments were performed during daytime (from 13:00 to 21:00) in autumn. The experimental period was divided into 3 intervals: 13:00-15:00, 16:00-18:00, and 19:00-21:00. The rats were elevated in a pressure chamber to an altitude of 11.5 km above the sea level for 60 sec. The RAH was measured as the period of survival under hypoxic conditions, i.e., the time after the "ascent" until a reversible arrest of breathing, after which the animal was "descended" [4]. The data were analyzed statistically using Statgraphics

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and Statistica software. The Kolmogoroff—Smirnoff's test with Lilliforce [9] and Shapiro corrections was applied to determine the type of distribution. The values of 34, 50%, and 66% quantiles were determined for each time-specific SP distribution to evaluate the number of LR, MR, and HR rats. The SP values in LR rats were equal or below 0.34, ranged from 0.35 to 0. 66 in MR rats, and were equal or above 0.67 in HR rats. Thus, the 0.34 and 0. 66 quantiles divided the region of possible SP values into 3 parts with approximately equal probability (about 30%) of falling into them for any particular value (Table 1).

Nonparametric one-way analysis of variance, median criterion, and Kruskal—Wallis test were applied to analyze daily variations in RAH. The differences between group medians and time-specific SP distributions were assessed by Mann—Whitney and Kolmogoroff—Smirnoff's tests, respectively. Parametric one-way analysis of variance was used to compare the group mean values for overall time period (with Fisher's test). The group means for pairs of intervals were compared using the least significant difference (LSD) test and analysis of variance (with Bartlett's test). Mann—Withney *U*-test was used to compare the group medians and means, as well as the number of LR, MR, and HR rats (as percent of total number) at specific time intervals. Pearson's χ^2 test was applied to compare the group sizes at different time intervals. In all tests the null hypotheses were rejected at the significance level of α=0.05.

RESULTS

A log-normal distribution of the population SP values was found within all time intervals ($p>0.05$), and the hypothesis of normal distribution was rejected at $p<0.01$. Although the two types of distribution were similar at 13:00 ($p<0.2$), the coefficient of asymmetry significantly exceeded the critical value at 5% significance level (data not shown), which excluded normal distribution. Because of distribution asymmetry the median was taken as its center [5]. The rat population showed considerable variations in SP values throughout the examination period (13:00-21:00), but not within specific time intervals.

At the same time, the SP values in rats with different RAH varied throughout the examination period and its 3 time intervals, as well as within specific intervals (at the beginning of the day in LR and MR and at the end of the day LR and HR). Paired comparison of the group SP values at different intervals (Fig. 1) and times (Fig. 2) showed the oscillations of RAH with a gradual decrease in its value at the end of the day. Paired comparison of the logarithms of SP values at different times also showed a decrease in SP by the end of the day. Correspondingly, the number of animals with SP below 50 sec increased in the middle (1.9 times) and especially at the end of the day (2.5 times), while the number of animals with SP values from 55 to 120 sec decreased at the end of the day (1.4 times, $p<0.025$), which agreed with changes

TABLE 1. Number of Rats with Low, Moderate, and High Resistance to Hypoxia at Different Time Periods (Percent of Total Number)

Time period, h	Total number of rats	Low-resistant		Moderate-resistant		High-resistant	
		n	%	*n*	%	*n*	%
Beginning of day							
13.00	11	4	36.36	4	36.36	3	27.28
14.00	26	10	38.48	8	30.76	8	30.76
15.00	31	14	45.16	8	25.81	9	29.03
Total...	68	28	41.18	20	29.41	20	29.41
Middle of day							
16.00	56	19	33.93	19	33.93	18	32.14
17.00	48	17	35.42	17	35.42	14	29.17
18.00	63	20	31.75	24	38.10	19	30.15
Total...	167	56	33.53	60	35.93	51	30.54
End of day							
19.00	59	19	32.20	20	33.90	20	33.90
20.00	42	14	33.33	16	38.10	12	28.57
21.00	38	13	34.21	9	23.68	16	42.11
Total...	139	46	33.10	45	32.37	48	34.53

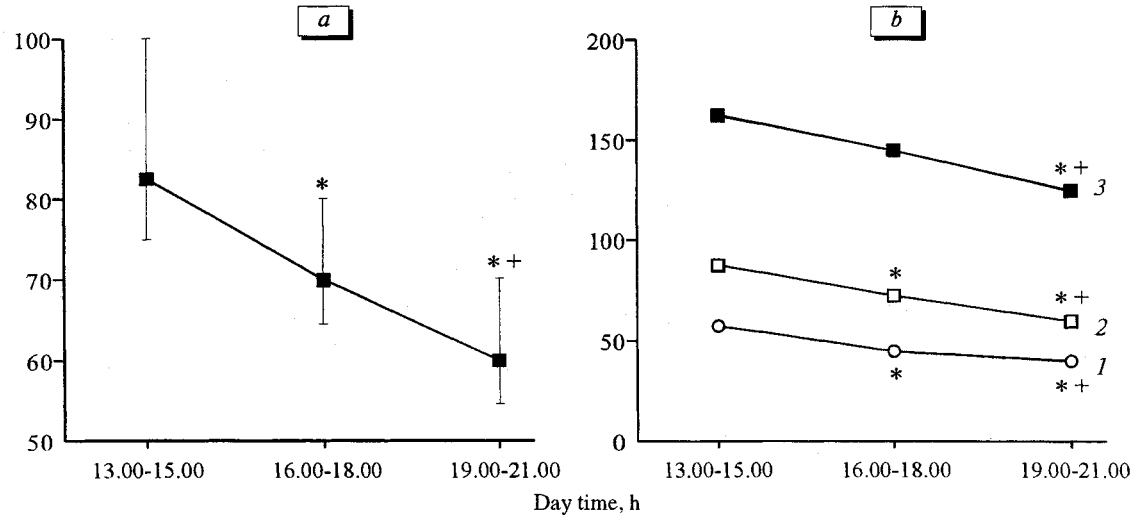

Fig. 1. Median values of survivial (sec) during three intervals of daytime. p=0.027-0.1×10^{-6}: *in comparison with 13:00-15:00; +in comparison with 16:00-18:00 h. Mann—Whitney test. Here and in Fig. 2; *a)* whole population; *b)* rats with low (*1*), moderate (*2*),and high (*3*) resistance to hypoxia.

in the SP distributions (Fig. 3). In LR and MR rats, RAH oscillations were similar to those in the whole population, being more pronounced (particularly in LR rats, Fig. 2). The maximum values of group and population medians of SP (at 13:00 and 15:00) 1.5-2 times surpassed its minimal values (at 20:00). In HR rats, the RAH value remained practically the same until 20.00 h and then decreased. In this group, the maximum median was almost twice as high as the minimal (at 21:00). In the majority of rats, different median values corresponded to different distributions. Thus, LR rats, the most stable RAH was observed from 16:00 to 19:00, in MR rats in the 16:00-18:00 and 19:00-21:00 intervals, and in HR rats from 13:00 to 20:00 . Therefore, all rats showed stable RAH from 16:00 to 18:00.

Due to diverse dynamics of RAH the intergroup difference in RAH varied throughout the day. The daily-average median value for HR group was twice as high as that for MR rats, the latter being 1.6 times higher than the corresponding value for LR rats. The average difference between the HR and LR groups was 3.3 times (from 1.9 to 4.2 at different times, p<0.01). The lowest intergroup difference was observed at 15:00, the highest at 20:00. Despite the different medians, the rows of SP values showed transgression (Table 2), which was 2 times higher for the LR and MR rows (63.5%), than for the HR and MR rows (30%). This fact together with similar diurnal dynamics of RAH indicates a similarity between LR and MR rats. Despite the low limit of HR row (at 21:00) was very close to the upper limit of LR row

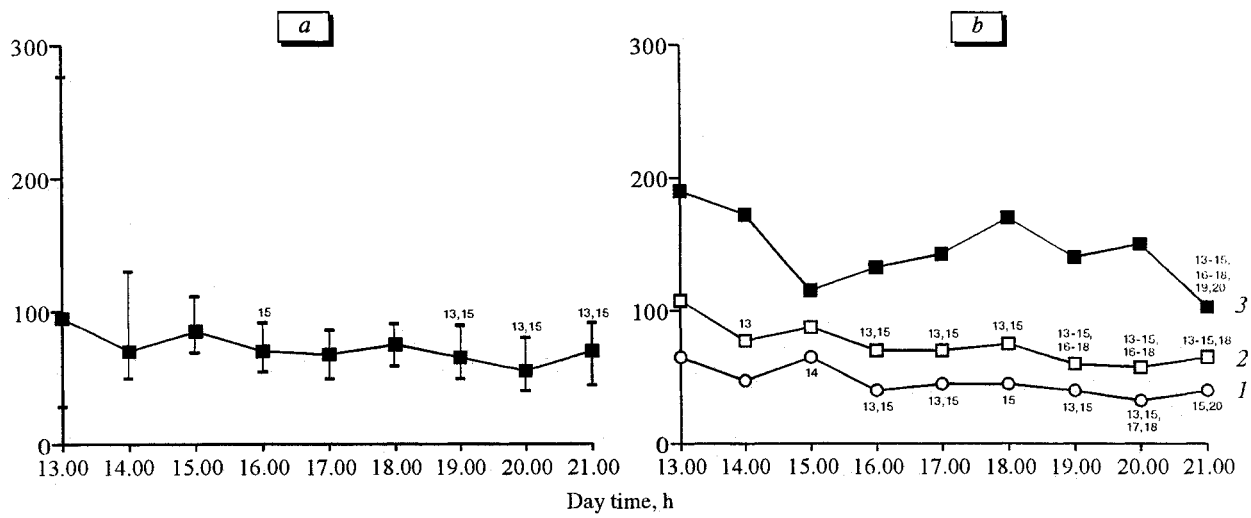

Fig. 2. Median values of survival (sec) as a function of daytime. The figures near the symbols: differences with indicated time points are significant (p<0.05-0.0001, Mann—Whitney test).

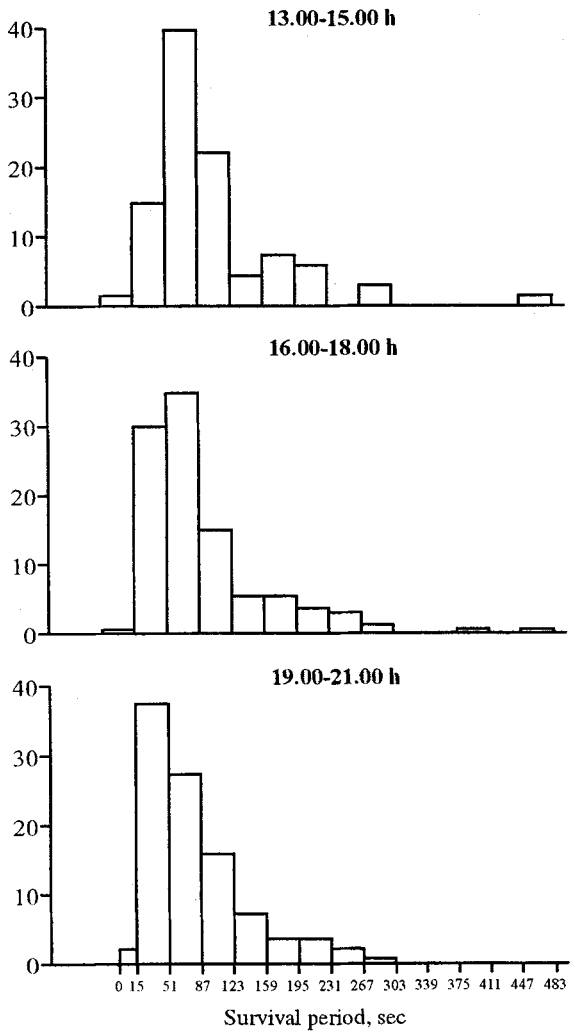

Fig. 3. Relative frequency of survival periods (%) for the whole rat population during daytime.

(13:00), SP rows of these groups showed no transgression.

For all time intervals, the group arithmetic mean exceeded the median value by 28.1%. In LR group, the geometric mean was 13.3% lower than the median ($p<0.05$).

The group size remained the same throughout the day (Table 1). At the same time, the group of LR rats was larger than both the MR (1.3 times at the first interval) and HR (1.24 times at the first and second intervals, 1.13 times on average) groups. These variations is probably due to differences between empirical and theoretical samples, which is evidenced by its highest value at the beginning of the day when the sample sizes were not as large as later on.

Table 2. Limits of SP Rows in Rats with Different Resistance to Acute Hypoxia (13:00-21:00)

Limits, sec	Low-resistant	Moderate-resistant	High-resistant
Minimum	10.0	44.8	82.3
Maximum	80.1	128.9	465.0

Thus, daily variations of SP values in Wistar rats, like daily and seasonal variations in outbred rats [1], were described by a log-normal distribution [1]. The log-normal distribution was typical not only of large (n=104-139), but also of relatively small (n=11-63) samples. Because of asymmetry, the center of distribution was most precisely characterized by the median [5]. The mean suggested for this purpose in [4] was higher than the median, while the geometric mean proposed in [1] was lower. The variations in the group size during the day (HR group was always the smallest) was probably due to the difference between theoretical and empirical distributions. Despite the similarity in distribution types, Wistar and outbred rats exhibited different diurnal dynamics of SP: at 13:00 Wistar and outbred rats showed the highest and the lowest RAH, respectively; at 19:00 RAH was relatively low in Wistar and reached the peak in outbred rats; Wistar rats maintained the same RAH value until 21:00 and outbred rats until 22:00 [2]. The peak values of RAH in Wistar rats were observed early in the day, which is in line with the data on mice showing that RAH is higher during light period than during dark time [13].

Our data showed that even inbred animals differ in not only RAH (with difference in values of up to 4.2 times), but also in its diurnal dynamics. It is likely that both RAH and its variability, at least circadian, are genetically determined. The pineal gland and suprachiasmatic nucleus of the hypothalamus [15] which regulate circadian rhythms may underlie the sense of time (for instance, the recognition of dawn or twilight periods) in rats even in complete darkness [11]. Circadian rhythms are controlled by melatonin that is synthesized in the pineal gland (epiphysis) [15] from serotonin after noradrenaline-induced activation of β-adrenoceptors [14]. At the same time peculiarities of the noradrenaline and serotonin metabolism were demonstrated in the hypothalamus and other brain regions of rats with genetically different resistance to stress [6, 7,12], which implies that these rats can also differ in the melatonin metabolism. This suggestion is supported by increased resistance to stress after melatonin administration [14]. It can be also hypothesized that differences in RAH are determined by inherent distinctions in the metabolism of biogenic amines and serotonin. The synthesis of melatonin depends on light conditions and changes within 1 h after their modification [14]; therefore, the circadian rhythm of RAH can also be determined by melatonin. At the same time, RAH diversity can be related to some distinctions in bioenergetic processes in the brain, myo-

cardium [8], and liver [3], as well as to the relative activities of lipid peroxidation and the antioxidant systems of the myocardium [10]. Since epiphyseal melatonin triggers circadian rhythms in different tissues [15], these processes must undergo diurnal variations, which predetermine the corresponding changes in RAH.

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