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Seasonal Variations in Rat Resistance to Hypoxia

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Resistance to acute hypoxia was evaluated by the life span after elevation to a simulated altitude of 11,500 m at 13.00-21.00 in different seasons of the same year. The period of investigation was divided into 3 parts: beginning (13.00-15.00), middle (16.00-18.00), and end (19.00-21.00) of the day. Animal life span decreased by the end of the day (the decrease was more pronounced in summer and autumn) and by the end of the year, *i. e.* in autumn (more pronounced in the middle and end of the day). The life span was longer in the middle of the day in spring in low- and medium-resistant rats and by the end of the year in medium-resistant rats (at the beginning of the day). Fluctuations in the life span during the day and year were more expressed in low- and medium-resistant rats. Differences in the life span of highly and low-resistant rats were the most pronounced in winter.

Key Words: *resistance to hypoxia; day; season*

Resistance to acute hypoxia in low- (LR), medium- (MR), and highly resistant (HR) rats can be evaluated by the life span (LS) under conditions of acute hypoxia [3,4,7-9]. This parameter is determined by genotype and depends on environmental factors [3], primarily on the season [1] and time of the day [1,2]. However the effects of these factors on LS of inbred rats with different hypoxic resistance was never investigated.

We investigated changes in LS of rats with different resistance to acute hypoxia during various seasons at different time of the day.

MATERIALS AND METHODS

Male Wistar rats (150-180 g, *n*=1144) were elevated in a pressure chamber to a simulated altitude of 11,500 m above sea level during 60 sec. Experiments were carried out at 13.00-21.00 in different seasons (Table 1). The time of investigation was divided into 3 periods: beginning (13.00-15.00), middle (16.00-18.00), and end (19.00-21.00) of the day. Resistance to acute hypoxia was evaluated by the duration of life in a pressure chamber after elevation until reversible respiration arrest, after which the animals were descended [3]. In order to detect the range of LS for each hour of the day, the type of LS values distribution was determined using Kolmogorov—Smirnov's consensus test (with Lilliforce supplement) and Shapiro—Wilks' test, after which 34 and 66% quantiles ($C_{0.34}$ and $C_{0.66}$) were estimated for the resultant distribution. We assumed that LS of LR rats was no more than $C_{0.34}$, that of MR rats between $C_{0.34}$ and $C_{0.66}$, and of HR rats more than $C_{0.66}$, *i. e.* the probable LS range was divided into 3 parts (ranges), the probability of distribution of the values in each of these ranges being about 30% (Table 1). The median served as the characteristic of LS distribution for the rats of all groups [9]. Nonparametrical methods were used: bifactorial dispersion analysis and Dunn's method of multiple comparisons for evaluating the relationship between time of the day and season, on the one hand, and rat LS, on the other, and Pearson's χ^2 test for comparing the incidence of the de-

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tected distribution type in the rats. The zero hypotheses were rejected at α=0.05.

The results were statistically processed using Statistica 5.0 software.

RESULTS

Right-sided asymmetry of LS distribution was observed in all rats during all seasons. The distribution was log-normal in 86% (different hours of the day) and 58% (different periods of the day). In other cases the distribution was not identified (including cases when the study was carried out during the whole day), *i. e.* their number increased with increasing studied interval (p<0.05). This can be explained by superposition of distributions. In rats with different resistance LS distribution was also asymmetrical: left-sided asymmetry was most typical of LR rats and right-sided asymmetry of HR and MR rats.

The season was an essential factor for LS in all rats (p<0.01); season and time of the day (period and hours) were significant for rats with different resistance to hypoxia, the time course of LS during the day being different in different seasons (p<0.01). During all seasons LS varied during the day and gradually decreased by the end of the day; maximum LS being determined for all periods of the day. LS was most stable in winter; its peaks were observed only in MR rats at 14.00 and 20.00. For LR rats the maximum LS was observed in summer at 13.00-16.00 and 21.00 and in autumn at 15.00. In autumn LS of all rats decreased by the end of the day (Fig. 1). In spring the pattern of LS changes was different: maximum at 18.00 in LR and MR rats and minimum at 17.00 in HR rats, LS being virtually the same in all groups of animals at the beginning and end of the day. It is noteworthy that LS changed during long period and within one hour. Fluctuations in LS during the day in all seasons were more pronounced in MR and LR rats and minimum in HR animals (p<0.05). Seasonal differences in LS were detected for all periods of the day (Table 2). In the majority of cases LS of all rats decreased by the end of the year, being shorter in autumn than during other seasons. Seasonal differences were more pronounced at the end of the day and most of all in MR rats (MR>LR>HR in the order of decrease in the frequency of differences, p<0.05-0.01).

LS was virtually the same in winter and spring; only in MR rats it was longer at the beginning of the day in winter than in spring. One more trend of LS changes (its increase during the year) observed in MR rats was as follows: LS longer in autumn than in spring at the beginning of the day and longer in spring than in winter in middle of the day. Evaluation of the seasonal differences in the rat LS during the whole day (13.00-21.00, Fig. 2) also showed its decrease during the year. LS of all rats was longer during all seasons than in autumn; according to designations in Table 2, the life span of MR rats in different seasons was as follows: W=Sp>S>A and W>S. This determined 1.1-2.7-fold differences in rat LS during the day and in different seasons (p<0.05-0.01). LS differences in rats of different groups also varied during the day and year. The difference in LS of HR and LR rats was more pronounced in winter (up to 4.6 times) than in summer and autumn (almost 3-fold) during all periods of the day (p<0.05). The range of fluctuations in LS difference between HR and LR rats was maximum in autumn (from 1.8 at 15.00 to 5-fold at 20.00). The mean ratio of LS of LR:MR:HR rats was 1:1.6:3.2, MR rats more markedly differed by LS from HR rats (2.1 times) than from LR rats (p<0.05-0.01). This reflected more significant transgression of LS series for MR and LR (81.4%) than for MR and HR (47.8%) for the whole year (p<0.01).

In some studies resistance to acute hypoxia was higher during daytime than during nighttime. During the day (12.00-21.00) the resistance to hypoxia decreased in random-bred rats exposed to hypoxic hypoxia in summer [1] and autumn [2], in mice (hypercapnic hypoxia) by midday in spring [2] (like in HR rats), in summer, and after injection of myorelaxine in autumn [2]; in winter and spring resistance to acute hypoxia in random-bred rats did not change [1], similarly to that in Wistar rats. On the other hand, in spring the resistance of random-bred rats decreased during the day (like in mice after myorelaxine injection) [2] and increased in summer [2] and autumn [1] (like in mice

TABLE 1. Distribution of Rats Tested during Daytime (13.00-21.00) in Groups with Different Resistance to Acute Hypoxia

Seasons	All rats	LR		MR		HR	
		n	%	*n*	%	*n*	%
Winter	119	46	38.7	39	32.8	34	28.6
Spring	206	76	36.9	69	33.5	61	29.6
Summer	443	163	36.8	142	32.1	138	31.2
Autumn	376	136	36.2	121	32.2	119	31.7

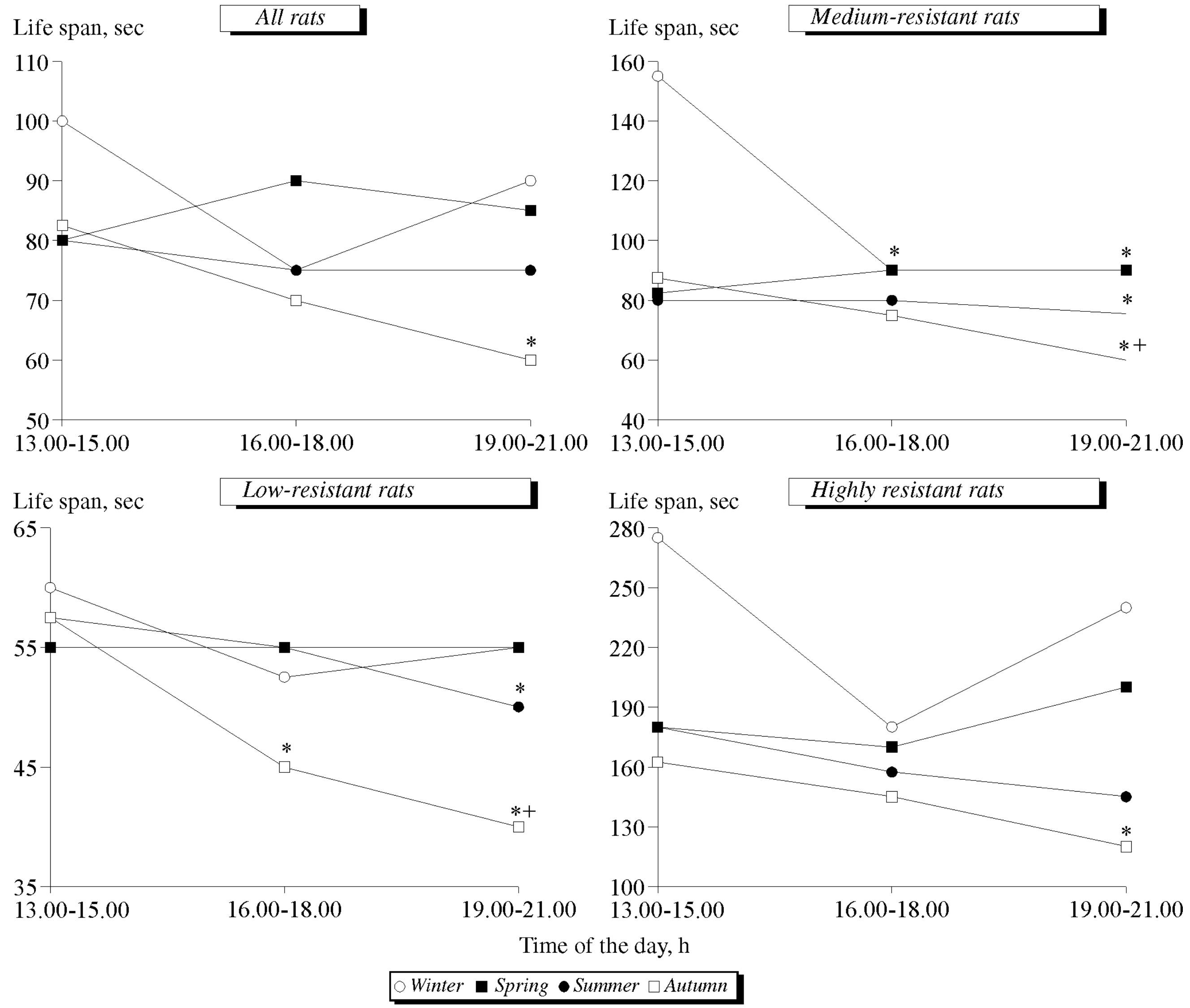

Fig. 1. Changes in the rat life span median during three periods of the day in different seasons. p<0.05-0.01 (Dunn's test): in comparison with *13-00-15.00, +16.00-18.00.

with hypercapnic hypoxia [2]). Differences in the time course of resistance during one season can be due to species or strain characteristics of animals or due to natural factors of different intensity in different years of investigation.

Let us characterize in brief the mechanisms underlying changes in animal resistance. The phenomenon of cross-adaptation to long-term stress and hypoxia is well known [6]. We can expect that urgent adaptation, the essence of resistance to acute hypoxic and stress exposures, is also cross-type. A negative relationship of resistance to acute hypoxia (in random-bred rats [2]) or stress resistance (in rabbits [10]) to locomotor activity was detected. Decreased stress resistance during the beginning of locomotor activity is associated with the maximum increase in left-ventricular myocardial contractility [10]. Resistance to hypoxia is decreased during dark hours in the twilight animals (Wistar rats). This was associated with increased locomotor activity and left-ventricular myocardial contractility [15]. The concentration of catecholamines [14] and corticosteroids [12] was increased in LR rats in the middle of the day (15.00-17.00) in spring and summer, while in HR rats the concentration of corticosteroids did not change during the whole day [4]. This could lead to more pronounced difference in the resistance of LR rats (in comparison with HR rats) during the day. Lipid peroxidation (LPO) and antioxidant system (AOS) also can modulate circadian and seasonal fluctuations of resistance to hypoxia. High activity of antioxidant enzymes in the morning and its decrease (along with impaired resistance to oxidative stress) were detected in rat myocardium by 19.00 [11], which corresponded to decreased rat resistance to hypoxia in

TABLE 2. Seasonal Differences in Rat LS in Different Periods of Day

Day period	All rats	Rat group		
		LR	MR	HR
Beginning	W=Sp=S=A	W=Sp=S=A	W>(Sp=S=A)	W=Sp=S=A
Middle	W=Sp>(S=A)	(W=Sp=S)>A	W<Sp>S>A	W=Sp=S=A
End	(W=Sp=S)>A	W=Sp>S>A	W=Sp>S>A	(W=Sp=S)>A

Note. W: winter; Sp: spring; S: summer; A: autumn; no difference between W and S in LR rats; W>S during all periods of the day and W<A at the beginning of the day in MR rats; W>A in LR and MR rats.

the evening. LPO activation and inhibition of antioxidant activity (in the liver and erythrocytes of Wistar rats) were observed during the twilight period in summer, which can be attributed to metabolism activation during motor activation, corticosteroids exerting a synchronizing effect on LPO and AOS activity [5]. More pronounced seasonal changes in cardiac LPO activity and AOS [7] were detected in LR rats in comparison with HR rats, the pattern of changes in the groups being different: virtually similar hypoxic resistance in LR rats in winter and summer was associated with a decrease in LPO and AOS activity and increased index of AOS functioning (LPO/AOS), while in HR rats AOS activity increased by winter. Greater differences in hypoxic resistance correlated with greater differences in LPO and AOS values in LR and HR rats in winter in comparison with summer: in summer LPO and AOS of the heart were similar in these two groups [7], while in winter liver LPO activity and LPO/AOS in the heart and liver were higher in LR rats [8]. Differences in hypoxic resistance of LR and HR rats were most pronounced during functional strain of organs (in winter) and little expressed during functional rest in summer. Circadian and seasonal changes in LPO and AOS activities can directly depend on pineal hormone melatonin, transmitting photoperiodic information for organization of circadian and seasonal rhythms. In addition, this hormone acts as a trap for free radicals, this lipophilic hormone penetrates cell membranes and activates many antioxidant enzymes in cells [13], which can improve animal resistance to hypoxia.

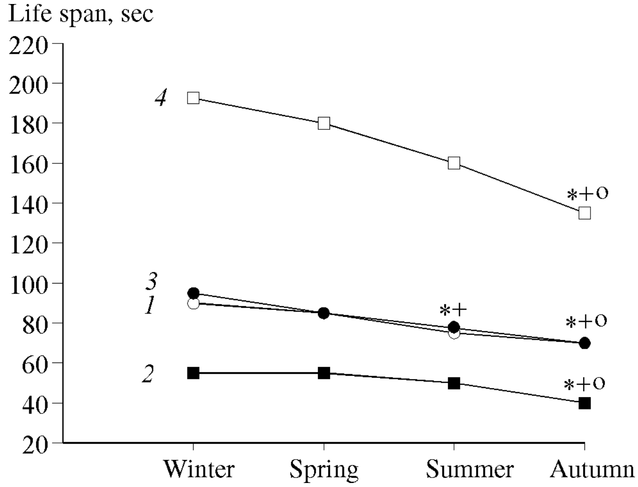

Fig. 2. Median of rat life span during the year. *1*) all rats; *2*) low-, *3*) medium-, and *4*) highly resistant rats. p<0.05-0.01 (Dunn's test): *compared to winter, +compared to spring; °compared to summer.

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