EXPERIMENTAL BIOLOGY

Effect of the Season on Lipid Peroxidation in the Myocardium of Rats with Different Resistance to Hypoxia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 7, pp. 87-90, July, 1995
Original article submitted July 21, 1994

Lipid peroxidation and the antioxidant system of the myocardium of adult male Wistar rats with low and high resistance to acute hypoxia tested by "raising" to an altitude of 11.5 km are studied in winter and in summer. It is found that the winter season is a mild stressor inducing changes in the myocardial antioxidant system and lipid peroxidation which are similar to those observed at the early stages of catecholamine stress in the summer season. In both cases alterations are more pronounced in low-resistance than in high-resistance rats. In winter, in low-resistance rats the intensity of lipid peroxidation and the activity of the antioxidant system are lower, while the ratio of their parameters (chemiluminescence data) is higher. At the same time, the levels of thiobarbituric acid-reactive substances are higher in winter in both groups. The relationship between the studied parameters and the resistance of rats to hypoxia is more obvious in winter than in summer, i.e., it is season-dependent and is also more pronounced in catecholamine stress.

Key Words: *lipid peroxidation; antioxidant system; hypoxia; low resistance; high resistance; season*

It is known that myocardial contractile function, the number and state of mitochondria [6,7], the number of lysosomes and the permeability of their membranes [7], and the activity of enzymes involved in anaerobic and aerobic metabolism of the myocardium and other organs [13] vary depending on the season. Seasonal variations have been observed for the blood level of fatty acids [6] and the activity of 3-hydroxyacyl-CoA dehydrogenase, an enzyme of their turnover in the liver [13]. Since energy and lipid metabolisms influence the intensity of lipid peroxidation (LPO) and the activity of the antioxidant system (AOS), it can be assumed that these characteristics are season-dependent. Lipid peroxidation is known to be involved in ischemic and reperfusion damage to the myocardium [11], which is season-dependent [14], and therefore, it is important to determine the relationship between LPO and AOS activity; yet such studies are scarce, and the myocardium of animals with different resistance to hypoxia has not been investigated. Our purpose was to study LPO and AOS in the myocardium of rats with low and high resistance to hypoxia in winter and in summer.

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MATERIALS AND METHODS

Male Wistar rats were tested in winter and in summer as described [1] by "raising" them to an

0007-4888/95/0007-0733$12.50

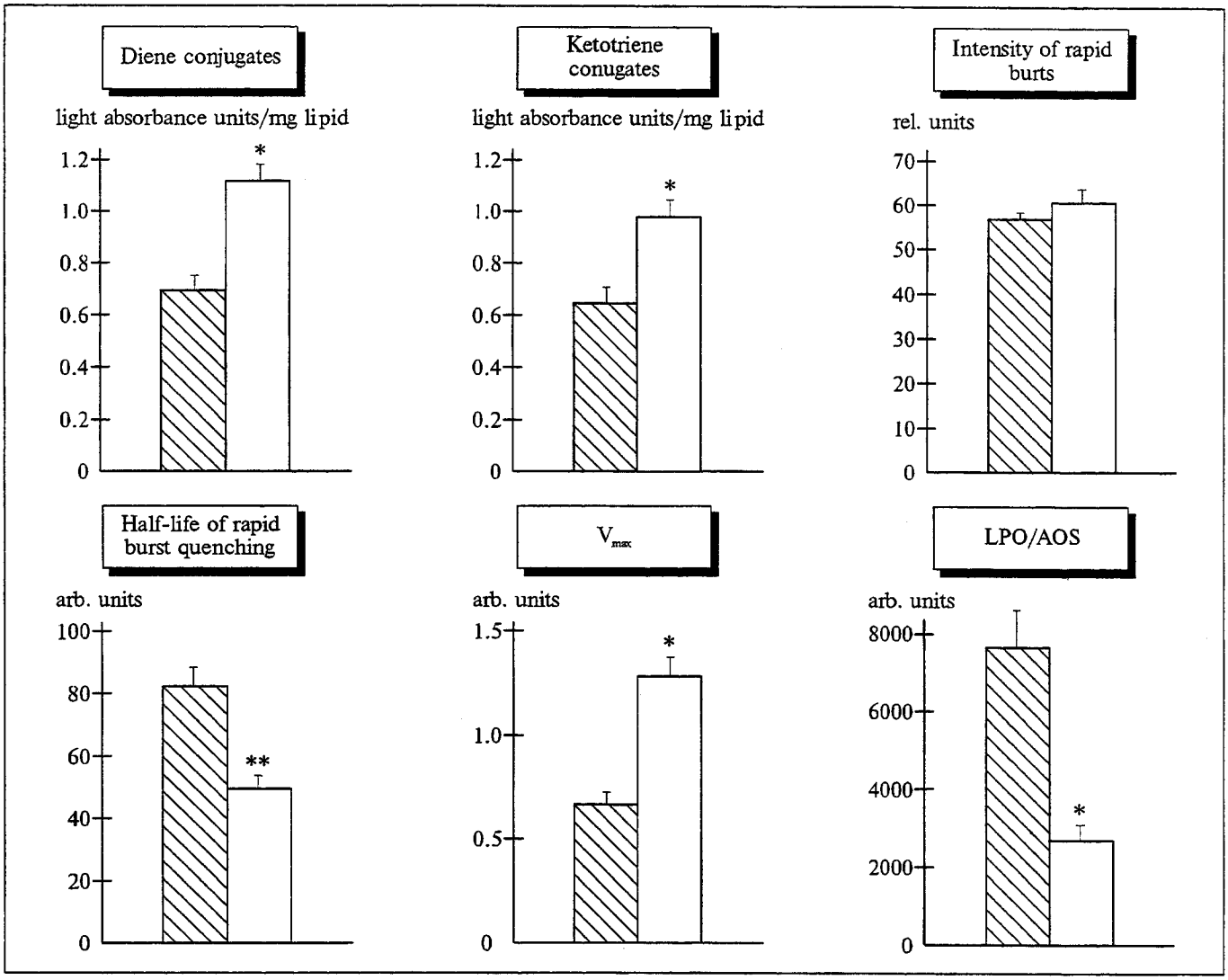

Fig. 1. Comparison of myocardial LPO and AOS parameters of LR (shaded bars) and LR (white bars) Wistar rats in winter (four samples). One asterisk indicates $p<0.05$, two asterisks indicate $p<0.01$.

altitude of 11.5 km during 1 min (twice, three weeks apart). In winter, the survival period of high-resistance (HR) rats was 5.3-fold longer in comparison with low-resistance (LR) rats (450±42 *vs.* 86±5 sec) and 4.8-fold longer in summer (306±17 *vs.* 63±7 sec, $p<0.01$). Three weeks after the repeat test, the heart was incised under thiopental anesthesia after a 2.5-min perfusion with cold (0-4°C) normal saline. Lipids were extracted from the homogenate by a modified method [10] with a chloroform-ethanol mixture (1:2) in the presence of 10^{-5} M ionol. Light absorbance (D) of lipids dissolved in hexane was measured in a Hewlett-Packard spectrophotometer at 215 nm (total lipids), 232 nm (diene conjugates), and 275 nm (ketotriene conjugates). All parameters were calculated per mg lipid. The oxidation indexes D 232/215 and D 275/215 reflecting the proportion of lipids oxidized to diene and triene conjugates, respectively, were calculated. The content of LPO products reacting with 2-thiobarbituric acid (TBA-reactive products) in the homogenate was determined by a method described elsewhere [3] with modifications: malonic dialdehyde and hydroperoxides were measured in the presence of 10^{-2} M Fe^{2+} (Fe-malonic dialdehyde) and the rate of LPO induced with 10^{-5} M Fe^{2+} and 8×10^{-3} M ascorbate was assessed from the accumulation of malonic dialdehyde. The following parameters of Fe^{2+}-induced chemiluminescence were determined in the homogenate [4]: the intensity of the rapid burst, which reflects the content of endogenous hydroperoxides, the maximum rate of rapid burst inhibition (V_{max}), and the half-life of rapid burst quenching. The integral parameter of chemiluminescence that reflects the relationship between the activity of LPO (rapid burst intensity) and AOS (V_{max}/half-life of quenching) (LPO/AOS), i.e., the tension of AOS

function, was calculated. The protein concentration in the homogenate was determined by the biuret method. Results were statistically analyzed using the Wilcoxon-Mann-Whitney test and Student's t test. Correlation analysis was performed with an ES-1055 computer.

RESULTS

The resistance of LR and HR rats to acute hypoxia in winter was 35% ($p<0.02$) and 47% ($p<0.01$) higher, respectively, than that in summer. Seasonal changes in resistance to hypoxia were associated with seasonal variations of the light absorbance of lipids and of LPO and AOS activity in the myocardium. Some parameters were decreased in winter compared with summer: D 215 in LR and HR rats (1.3-fold, $p<0.05$) and the activity of LPO (intensity of rapid burst) and AOS (half-life of rapid burst) in LR rats. In LR rats LPO/AOS was higher in winter than in summer (Table 1). In addition, the content of TBA-reactive products in the myocardium of rats of both groups and AOS activity in HR rats were higher in winter than in summer. Seasonal differences in LPO and AOS in both groups were accompanied by variations of the relationship between myocardial LPO and AOS and resistance to hypoxia. For example, in summer all the studied parameters were practically the same in both groups, and a relationship between LPO parameters and resistance to hypoxia was revealed only by correlation analysis. In HR rats, there was a positive correlation between the survival period and D 275/215 ($r=0.773$, $p<0.05$). In LR rats, the activity of AOS was low: a negative correlation was established between the survival period and half-life of rapid burst quenching ($r=-0.589$, $p<0.05$). The higher intensity of LPO in HR rats and the lower activity of AOS in LR rats, which were less pronounced in summer, were more obvious in winter, when significant changes between LR and HR rats were recorded. In winter, the contents of diene and ketotriene conjugates and AOS activity were higher, while LPO/AOS was lower in the myocardium of HR than in LR rats (Fig. 1).

Changes in LPO and AOS activity in the myocardium recorded in winter in comparison with summer and the appearance of changes between the parameters of HR and LR rats in winter are similar to the changes in the parameters observed in both groups during catecholamine stress in summer [8]. For example, in catecholamine stress D 215 of myocardial lipids decreased by the 6th h in HR rats and in both groups by winter. A decrease in LPO intensity analogous to the changes developing after 1 h of catecholamine stress was recorded. However, the seasonal changes in the concentrations of TBA-reactive products differed from those occurring in stress: in winter the content of these products increased considerably in both groups, while in stress it decreased in LR rats (malonic dialdehyde) and in HR rats (Fe-malonic dialdehyde). The activity of AOS fell in stress in both groups and in LR rats in winter, which was accompanied by an increased tension of AOS function in LR rats in both cases. As already mentioned, in summer the LPO and AOS parameters did not differ in LR and HR rats, but in catecholamine stress [8] and in winter a difference similar in both cases was observed: the LPO concentration was higher in the myocardium of HR rats. In stress and under the influence of the seasonal factor changes in LPO and AOS were more pronounced in LR rats, while in HR rats the parameters were more stable. At the same time, the seasonal factor induced changes in a lesser number of parameters compared with stress. From this it can be concluded that winter is a relatively mild stressor inducing shifts in the activity of myocardial LPO and AOS. This conclusion is consistent

TABLE 1. LPO and AOS Parameters in the Myocardium of LR and HR Wistar Rats in Summer and Winter ($M\pm m$)

Season	TBA-reactive products			Chemiluminescence parameters			
	malonic dialdehyde, pmol/mg protein	Fe-malonic dialdehyde, pmol/mg protein	Fe-ascorbate-malonic dialdehyde, pmol/mg protein×min	intensity of rapid burst, rel units	half-life of quenching, arb units	V_{max}, arb. units	LPO/AOS, arb. units
LR							
Summer (7–10)	7.66±0.35	6.02±0.52	0.316±0.105	63.0±1.1	57.4±3.5	0.925±0.116	3864±622
Winter (4)	15.0±3.0*	40.0±3.0**	1.120±0.511	56.7±2.4*	82.3±4.1**	0.665±0.110	7628±2092*
HR							
Summer (6–7)	7.42±0.92	6.78±0.87	0.420±0.144	65.8±4.3	61.3±2.9	1.097±0.149	3884±503
Winter (4)	13.0±1.0**	41.0±5.0**	0.203±0.095	60.5±4.8	49.5±4.8*	1.280±0.250	2686±739

Note. *$p<0.05$; **$p<0.01$ between indexes in summer and winter. The number of samples is given in parentheses.

with the findings that in mammals the functional tension of the myocardium is maximal in winter and minimal in summer [6,7]. Presumably, winter is also a stressor for coldblooded animals, inducing, for example, hypoxia in fish [13] and lactate acidosis in turtles [15]. The different shifts in LPO and AOS caused by catecholamine stress and seasonal factors probably stem from the specific features of the stressors. The marked decrease in AOS activity observed in LR rats in winter may result from a lower consumption of naturally occurring antioxidants. This assumption is confirmed by a decrease in the number of AOS components in rats maintained on an antioxidant-deficient diet [2,5,12]. On the other hand, such a diet activates a number of antioxidant enzymes [2], and this is probably what occurred in the myocardium of HR rats, in which the AOS activity increased. The shifts in AOS are accompanied by changes in the plasma membrane phospholipids and their oxidizability [9], which indirectly reflects the season-related decrease in light absorbance of myocardial lipids parallel to changes in the content of LPO products.

Thus, it is found that winter (in comparison with summer) is a mild stressor inducing shifts in the activity of myocardial LPO and AOS which are more pronounced in LR than in HR rats. The relationship between the intensity of LPO and activity of AOS in the myocardium on the one hand and the resistance to hypoxia on the other is most obvious under the influence of a mild stressor, for example, winter, i.e., it is dependent on the season.

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