Lipid Peroxidation Parameters in the Internal Organs of Rats with Different Degrees of Resistance to Hypoxia

M. L. Khachatur'yan, V. M. Gukasov, P. G. Komarov, L. B. Pirogova,* and M. V. Bilenko**

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Lipid peroxidation and the antioxidant system of the heart, liver, and brain are studied in adult male Wistar rats with high and low resistance to hypoxia tested by "raising" to an altitude of 11.5 km and in intact outbred rats. These parameters are found to be the same in the brain of low- and high-resistance rats, while the brain content of lipid peroxidation products is higher in both groups of Wistar rats compared with outbred rats. The heart and liver parameters are coupled to the resistance to hypoxia. Antioxidant activity prevails over lipid peroxidation in the hearts and livers of high-resistance rats, confirming that oxidation plays a major role in the damaging and lethal effects of acute hypoxia.

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

Hypoxic and reoxygenation damage are known to be associated with diminished activity of the antioxidant system (AOS) [12,13] and with activation of lipid peroxidation (LPO) [2,12, 13]. Adaptation to hypoxia leads to changes in the activity of tissue AOS [3,8] and in the content of LPO products [6,7]. At the same time, species-specific and intraspecies (individual) variations of resistance to hypoxia have been demonstrated [1]. It can be assumed that the activity of tissue AOS and LPO is different in animals with different degrees of resistance to hypoxia. Meanwhile, few studies have been performed on rat blood [10], while the state of LPO and AOS in the vital organs in animals differing in their resistance to hypoxia has not been assessed. Our purpose was to investigate the LPO content and AOS activity in the brain, heart, and liver of rats with low and high resistance to hypoxia.

Department of Experimental Biological Models and *Department of Mathematical Methods, Russian State Medical University; **Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow (Presented by A. I. Archakov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were performed on 20 Wistar rats and 10 outbred rats in winter. Wistar rats were tested by "being raised" them to an altitude of 11.5 km during 1 min (twice, three weeks apart) [1]. 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, respectively, $p<0.01$). Three weeks after the repeat test, the brain, heart, and liver were rapidly excised under thiopental anesthesia after a 2.5-min perfusion with cold (0-4°C) normal saline. The organs were homogenized and homogenates prepared from organs of 2-3 animals were pooled in one sample. There were 4-5 samples in each group of rats. Lipids were extracted from the homogenates as described elsewhere [11] 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 D232/215 and D275/215 reflecting the proportion of lipids oxi-

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dized to diene and triene conjugates, respectively, were calculated. The content of LPO products reacting with 2-thiobarbituric acid (TBA-reactive products) was determined in the homogenates; the rate of LPO induced by 10^{-5} M Fe^{2+}, 0.8×10^{-3} M ascorbate, and 10^{-3} M NADPH [2] was determined in liver microsomes isolated by differential centrifugation. The following parameters of 10^{-2} M Fe^{2+}-induced chemiluminescence were determined in the homogenate [4]: the intensity of the rapid burst (I), which reflects the content of endogenous hydroperoxides, the half-life of rapid burst quenching (HLQ), and the maximum rate of rapid burst inhibition (V_{max}). The integral parameter of chemiluminescence, which reflects the relationship between the activity of LPO (I) and AOS ($V_{max}\times1/HLQ$)/(LPO/AOS), i.e., the tension of AOS function, was calculated. The protein concentration in homogenates and liver microsomes was determined by the biuret method. The results were statistically analyzed using the Wilcoxon-Mann-Whitney test. Correlation analysis was performed on an ES-1055 computer.

RESULTS

Comparison of LPO parameters in Wistar rats differing in resistance to hypoxia showed that in the heart the content of LPO products (diene and ketotriene conjugates) (Fig. 1) and AOS parameters (1/HLQ) are higher and LPO/AOS is lower (Fig. 2) in HR than in LR rats. The intergroup differences of LPO parameters are consistent with the correlation analysis data: a strong positive correlation between the survival period and the indexes of heart lipid oxidation D232/215 and D275/215 was established in HR rats (r =0.987 and 0.967, p<0.05). In the liver, LPO activity (I) was lower, AOS (V_{max}) was higher, and LPO/AOS was lower (Fig. 2) in HR than in LR rats. These results agree with the correlation analysis data: a strong negative correlation between the survival period and D275/215, HLQ, and LPO/AOS (r =-0.987, -0.951, and -0.999, respectively, p<0.05-0.01) was established in HR rats. In liver microsomes, the rate of NADPH-induced LPO was twice as high in HR rats as in LR rats (0.21±0.02 and 0.10± ±0.02 nmol/mg protein×min, respectively, p<0.05). In the brain, LPO and AOS parameters were similar in HR and LR rats. A strong negative correlation was found between the survival period and LPO/AOS (r =-0.996, p<0.05). Thus, the heart and liver AOS activities and the rate of NADPH-induced LPO in liver microsomes are strongly coupled to the resistance to hypoxia, and these parameters are higher in HR rats than in LR rats.

Comparison of LPO and AOS parameters of intact outbred rats with those of tested Wistar rats showed that the LPO parameters in livers and hearts of outbred rats are similar to those of Wistar rats with the exception of D232/215, which was higher in hearts of outbred rats than in LR rats (Fig. 1). In these organs, AOS activity was lower and LPO/AOS higher in outbred rats than in HR Wistar rats (Fig. 2). In liver mi-

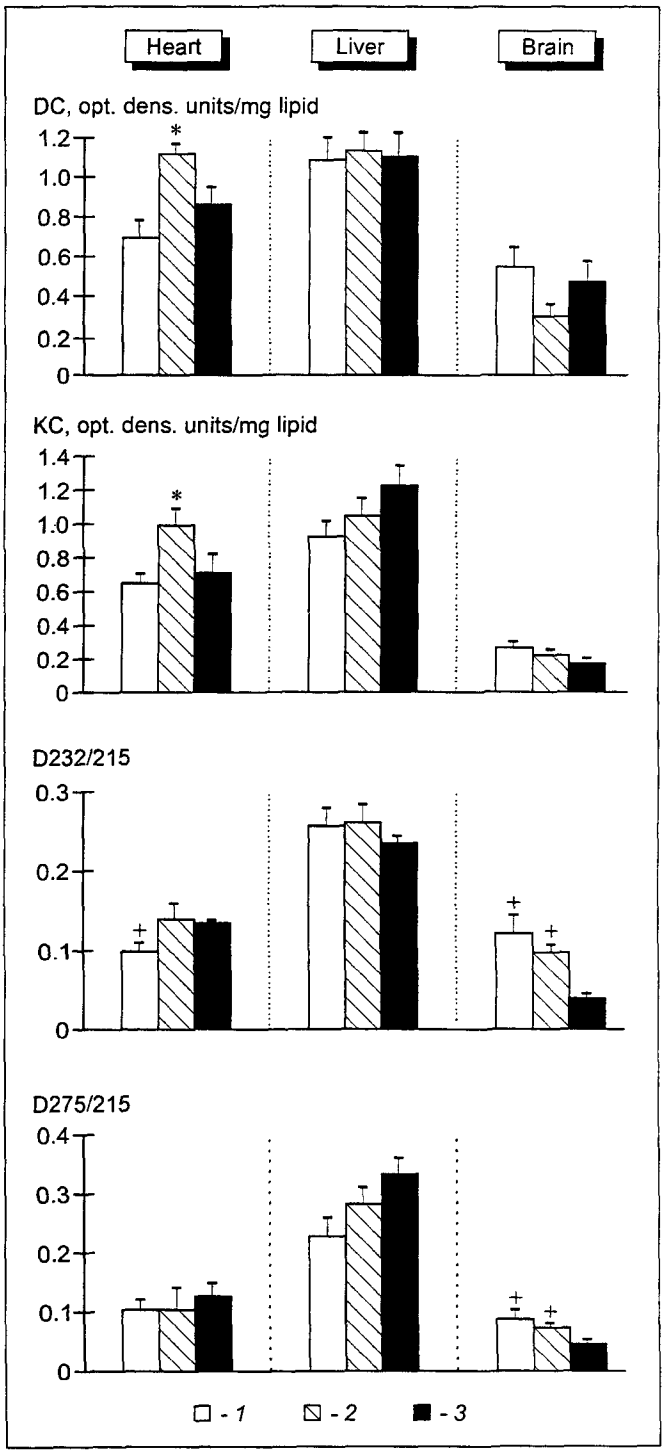

Fig. 1. Diene (DC) and ketotriene (KC) lipid conjugates in rat organs. Here and in Fig. 2: LR (*1*) and HR (*2*) Wistar rats and outbred rats (*3*). Significance of differences between parameters in LR and HR rats: *p<0.05; **p<0.01; significance of differences between LR and HR Wistar rats and outbred rats: +p<0.05, ++p<0.01.

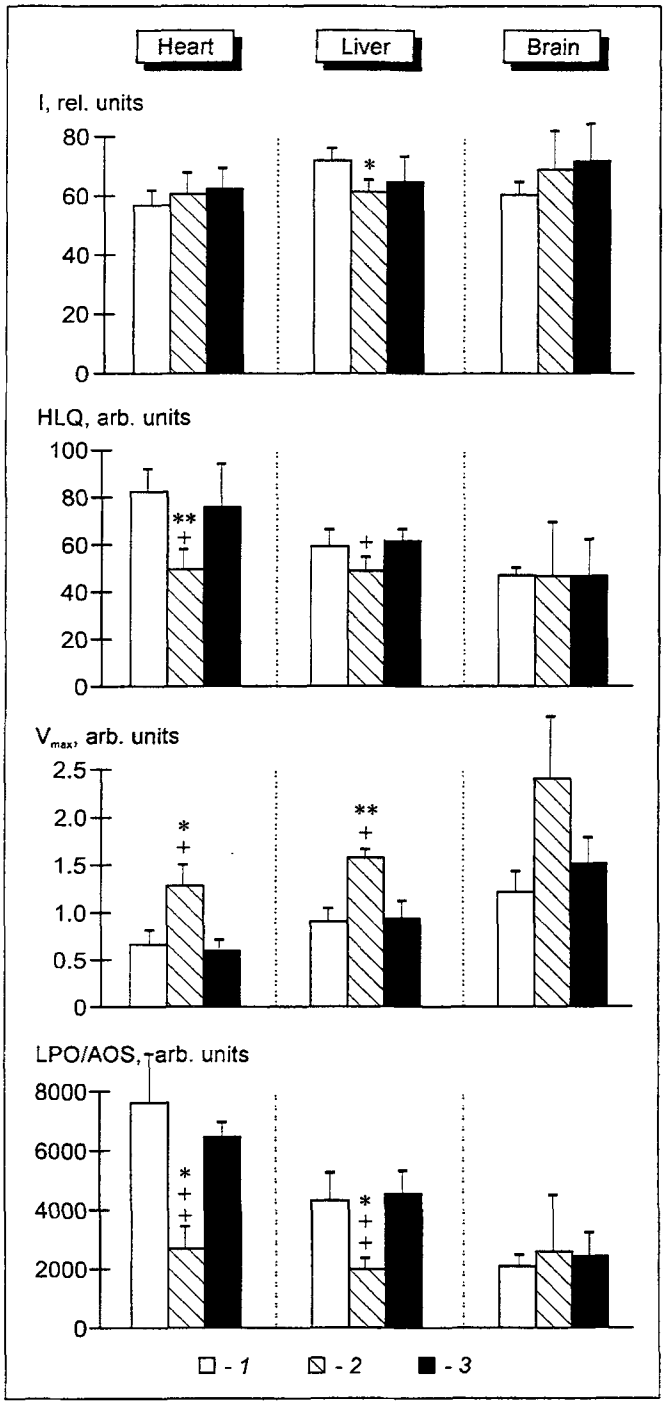

Fig. 2. Chemiluminescence parameters of rat organs.

crosomes, the rate of NADPH-induced LPO was 50% lower in outbred rats than in HR Wistar rats (0.09±0.04 and 0.21±0.02 nmol/mg protein×min, respectively, $p<0.05$). Since all these parameters of the heart and liver are coupled to the resistance to hypoxia, are practically the same in outbred and LR rats, and differ from the parameters in HR rats in the same manner, it could be assumed that these outbred rats had a low resistance to hypoxia. Meanwhile, the parameters of endogenous LPO in the brain were coupled to strain differences: in outbred rats, the indexes of oxidation of brain lipids were considerably smaller compared with those in HR and LR Wistar rats (Fig. 1). In all groups, the content of TBA-reactive products in the studied organs was practically the same (data not shown).

Thus, our data obtained on rats with different degrees of resistance to hypoxia are consistent with those of other researchers. Adaptation to high-altitude hypoxia, physical exercise, and stress increases the functional activity of AOS [8]. For example, the activity of antioxidant enzymes increases in rat myocardium during adaptation to swimming [15], in the myocardium and liver during adaptation to stress [8], and in the liver during adaptation to high-altitude hypoxia [3]. An increase in the resistance to hypoxia and in the reoxygenation of the hypertrophied myocardium is associated with enhanced activity of myocardial antioxidant enzymes [12]. Since adaptation to physical exercise and hypoxia is attended by activation of transcription and translation processes in the myocardium [6], the possibility cannot be ruled out that AOS activity is higher in HR than in LR rats due to more active synthesis of the proteins of antioxidant enzymes. In addition, energy metabolism differs considerably in animals with different degrees of resistance. For example, the glycogen content is much higher in the myocardium of rats with high resistance to isoproterenol than in rats with low resistance to it [14]. The NADH-oxidase pathway is the major source of reducing equivalents of the oxidation chain in LR rats, while in HR rats it is the succinate oxidase pathway [5]. Transformation of the Krebs cycle aimed at the prevalence of the dicarboxylic component, which potentiates the accumulation of succinate, occurs in hypoxia. This is more pronounced in HR rats than in LR rats [1]. Presumably, the "more optimal" energy metabolism in HR rats can make for a higher activity of AOS than that in LR rats. However, it is known that administration of exogenous antioxidants (tocopherol acetate and propyl gallate) increases the concentration of naturally occurring antioxidants in the liver, attended by increased antioxidant activity and lipid oxidation [9]. In the liver of HR rats, the higher activity of AOS goes along with higher lipid oxidation, which is probably associated with the higher rate of NADPH-induced LPO in microsomes compared with that in LR rats. In the heart of HR rats, the relatively high activity of AOS is probably coupled to the accumulation of readily oxidized lipid fractions. This hypothesis is based on the increase of the phosphatidylserine content in the cardiomyocyte sarcolemma of animals adapted to physical exercise [16], phosphatidylserine being a readily oxidized phospholipid [9]. This may promote a higher intensity of LPO in the myocardium of rats adapted to hypoxia compared with intact rats: an increase in the intensity of chemiluminescence [6],

an increase in the content of hydroperoxides and Schiff bases [7], and a higher content of diene and ketotriene conjugates in HR rats as compared with LR rats. In the brain of animals adapted to high-altitude hypoxia [3], the elevation of superoxide dismutase and glutathione peroxidase does not contradict our finding that AOS parameters are the same in the brain of HR and LR rats, since the total rather than discrete parameters of AOS were analyzed in this study.

Thus, our results indicate that in HR rats the AOS parameters are higher in the heart and liver, LPO/AOS (tension of AOS function) of these organs is lower, and the rate of NADPH-induced LPO in liver microsomes is higher than the corresponding parameters of LR rats. These liver and heart parameters coupled to the resistance to hypoxia may serve for a post-mortem estimation of resistance without a need for performing the two tests. The prevalence of AOS activity over LPO in the heart and liver of HR rats emphasizes the role played by LPO in the damaging and lethal effects of acute hypoxia.

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