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Comparison of Lipid Peroxidation Parameters in the Heart, Liver, and Brain of Rats with Different Degrees of Resistance to Hypoxia

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The relationship between the intensity of lipid peroxidation and the activity of the antioxidant system in the heart, liver, and brain is studied in male Wistar rats with low and high resistance to hypoxia tested by being "raised" to an altitude of 11.5 km and in intact outbred rats. It is found that in all groups of rats the content of lipid peroxidation products is highest in the liver, lower in the heart, and lowest in the brain. In all groups, the rate of the ascorbate-induced lipid peroxidation is highest in the brain, lower in the liver, and lowest in the heart. The activity of the antioxidant system is highest in the brain, lower in the liver, and lowest in the heart of low-resistance and outbred rats, while in high-resistance rats it is the same in all the organs. Thus, the difference in the parameters of lipid peroxidation and, particularly, of the antioxidant system in the studied organs is most pronounced in rats with a low resistance to hypoxia.

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

The brain is known to be the organ most sensitive to hypoxia, followed by the heart [8] and then the liver. In the heart, irreversible damage develops after 20-60 min of ischemia [15,16] and in the liver after 2-3 h [14]. The sensitivity of brain mitochondria to oxygen deficiency is higher than that of liver mitochondria [6]. However, in terms of the time of the initial significant increase in the content of lipid peroxidation (LPO) products in ischemia, these organs can be arranged as follows: brain (5 min)>liver (15 min)>heart (30 min) [2]. The different levels of sensitivity of these organs to ischemia may be associated with the initially different intensity of LPO and the activity of the antioxidant system (AOS) in them. In fact, the content of primary and secondary LPO products is highest in the liver, lower in the heart, and lowest in the brain; the antioxidant activity in the heart is considerably higher than that in the liver [2]. It can be assumed that the relationship between LPO intensity and AOS activity in the organs

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is also coupled to the resistance to hypoxia. Our objective was to assess the relationship between LPO and AOS in the heart, liver, and brain in low-resistance (LR) and high-resistance (HR) Wistar rats and in intact outbred rats.

MATERIALS AND METHODS

Experiments were performed on 20 adult male Wistar rats tested for resistance to acute hypoxia by being "raised" to an altitude of 11.5 km (the survival period of HR rats was 5.3-fold longer compared with LR rats) and on 10 intact outbred rats. Three weeks after the repeat test, the brain, heart, and liver were excised under thiopental anesthesia after perfusion with cold normal saline. The organs were homogenized, and the homogenates prepared from the organs of 2 or 3 animals were pooled. There were 4-5 samples in each group. Lipids were extracted from the homogenate. The light absorbance of lipids dissolved in hexane was measured at 215 nm (total lipids), 232 nm (diene conjugates), and 275 nm (ketotriene conjugates) and calculated per mg lipids. The oxidation indexes were calculated as the ratios between absorbance at 232 and 215 nm (232/215) and at 275 and 215 nm (275/215). The contents of LPO products reacting with thiobarbituric acid (TBA-reactive products) were determined in the homogenates: endogenous malonic dialdehyde and hydroperoxides were measured in the presence of 10^{-2} M Fe^{2+} (Fe^{2+}-malonic dialdehyde); the rate of LPO induced with 10^{-5} M Fe^{2+} and 0.8×10^{-3} M ascorbate was determined from the accumulation of malonic dialdehyde. The following parameters of Fe^{2+}-induced chemiluminescence were determined: the intensity of the rapid burst (I), which reflects the content of endogenous hydroperoxides, the half-life of rapid burst quenching (HL), and the maximum rate of rapid burst inhibition (V_{max}). The integral parameter of chemiluminescence that reflects the relationship between the activity of LPO (rapid burst intensity) and AOS ($V_{max}\times1/HL$) (LPO/AOS), i.e., the tension of AOS function, was calculated. The protein concentration in the homogenates was determined by the biuret method. Results were statistically analyzed using the Wilcoxon-Mann-Whitney test and Student's *t* test. See our previous paper [11] for a detailed description of the procedure.

RESULTS

In all groups of rats, the absorbance of lipids extracted from the heart was highest, that of lipids extracted from the liver lower, and that of brain lipids the lowest (Fig. 1). In contrast to light absorbance, LPO intensity, AOS activity, and LPO/AOS in these organs depended on the resistance to hypoxia (Figs. 1 and 3). In Wistar rats (both HR and LR) the contents of primary and secondary LPO products were highest in the liver, lower in the heart (more parameters differed in LR rats than in HR rats), and lowest in the brain. At the same time, the contents of TBA-reactive products were different, being highest in the brain and virtually the same in the liver and heart (Fig. 2). In LR rats, the AOS parameters were highest in the brain, lower in the liver, and lowest in the heart (Fig. 3). In LR rats, the tension of AOS function (LPO/AOS) in the liver and heart was considerably higher than in the brain. In HR rats, the AOS parameters and LPO/AOS in the studied organs were practically the same, since the AOS parameters in the liver and heart were as high and the LPO/AOS parameters as low as those of the brain. In outbred rats, the LPO and AOS differences in different organs were similar to those in Wistar rats, the difference of AOS parameters being smaller compared with those in LR rats. The contents of LPO products (diene and ketotriene conjugates, lipid oxidation indexes) were highest in the liver, lower in the heart, and lowest in the brain (Fig. 1). However, the concentration of TBA-reactive products in the brain was higher than that in the heart and liver (Fig. 2). The activity of AOS in the brain was higher than in the heart, while the parameters of liver AOS did not differ significantly from those in the heart and brain. The activity of AOS in the heart was considerably lower than in the liver and brain (Fig. 3).

Despite the intergroup differences in the relationship between the content of endogenous LPO products and the activity of AOS in the studied organs, the differences among the organs in ability to induce LPO were similar (Table 1). In all groups of rats, the

TABLE 1. Rate of LPO Induced with Fe^{2+} and Ascorbate in Rat Heart, Liver, and Brain ($M\pm m$)

Organ	Fe^{2+}-ascorbate-malonic dialdehyde, pmol/mg protein×min		
	LR rats	HR rats	outbred rats
Heart	1.12±0.511	0.20±0.09	0.23±0.04
Liver	38.90±11.20*	21.60±4.80**	56.00±18.16*
Brain	80.00±11.00***	73.00±4.00****	75.00±16.00**

Note. *$p<0.05$; **$p<0.01$ compared with the heart parameters; *$p<0.05$; **$p<0.01$ compared with the liver parameters.

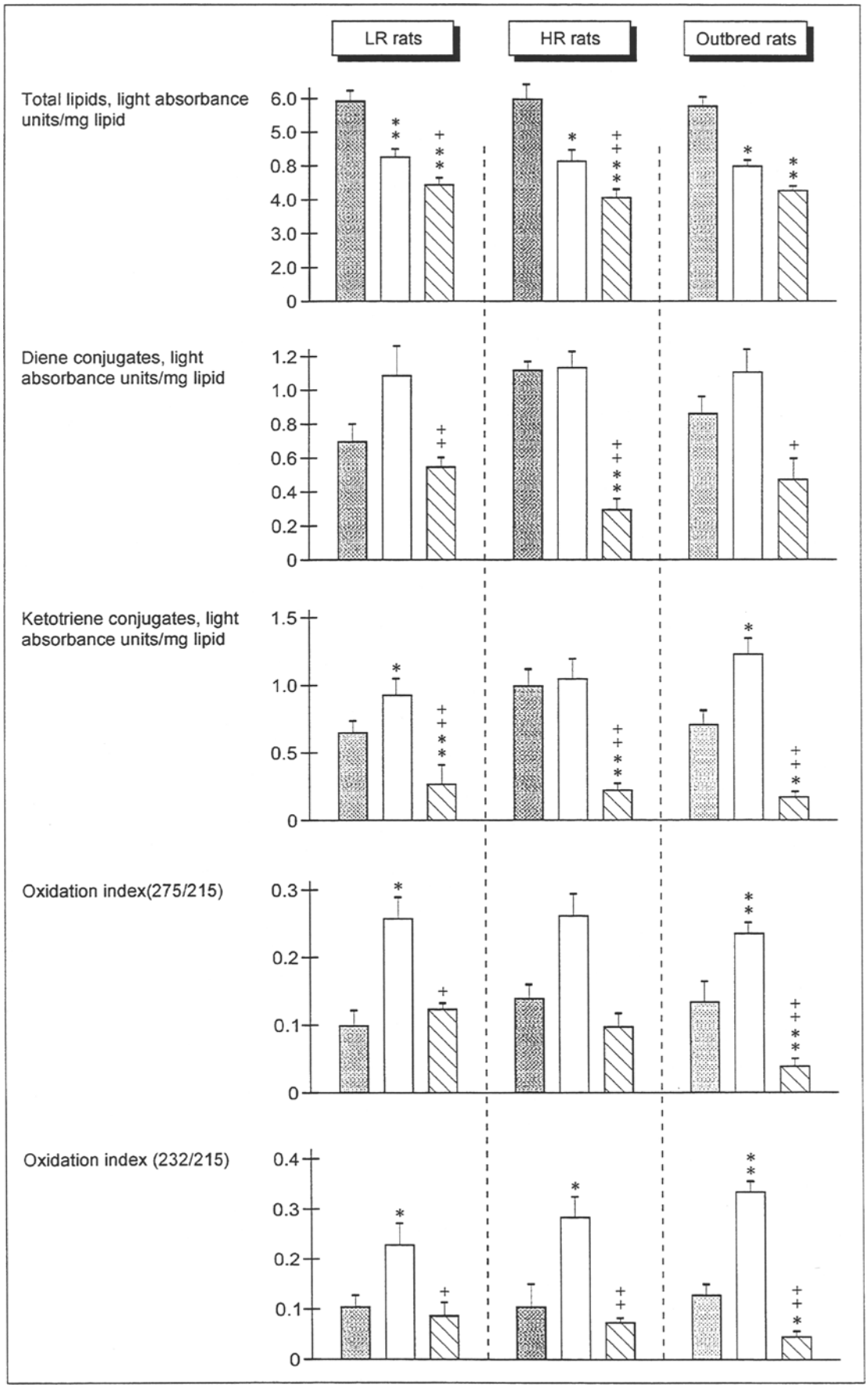

Fig. 1. Diene and ketotriene conjugates of lipids extracted from the heart, liver, and brain. Here and in Figure. 2 and 3: black bars represent the heart, white bars the liver, shaded bars the brain. $^{*}p<0.05$; $^{**}p<0.01$ compared with the heart parameters; $^{+}p<0.05$; $^{++}p<0.01$ compared with the liver parameters.

rate of ascorbate-induced LPO was highest in the brain and lower in the liver: however, in both brain and liver it was considerably (35- to 360-fold) higher than in the heart.

Thus, the relationship between the endogenous LPO parameters (with the exception of TBA-reactive products) in the heart, liver, and brain of LR and HR Wistar rats and outbred rats is consistent with the LPO-AOS relationships established in untested rats and rabbits [2,7,10]. Meanwhile, the ratio of the malonic dialdehyde content in the organs is known to be the same as the ratio of other LPO products in Wistar rats [2] and in rabbits (heart and liver) (A. A. Kubatiev, 1979). The differences in the rate of ascorbate-induced LPO and in the content of endogenous LPO products among the organs are similar in all groups of rats, the rate of ascorbate-induced LPO being maximal in the brain and minimal in the heart. However, there is evidence that the ability of brain tissue to induce LPO is 2- to 3-fold lower than that of the liver [13]. Our data agree with those obtained on rabbits: the period of LPO induction in the heart is almost twice as long as that in the liver. The differences in the ability to induce LPO may be due to a different capacity for oxidation of the lipids in these organs as a result of their composition. For example, the concentration of free fatty acids in the brain is 1.5-fold higher than that in the heart [3].

The different AOS activity in different organs demonstrated in the present study ties in with the fact that the antioxidant activity of brain lipids is considerably higher than that of heart lipids in rats [3] and heart and liver lipids in humans [4] and that catalase activity in the rat brain is higher (almost 2.5-fold) than that in the liver [1]. Being the reservoir of antioxidants, the brain displays the highest antioxidant activity [9]. Meanwhile, there are reports that the activity of glutathione peroxidase in the brain is substantially lower than in the heart in rats and in the liver (50- to 200-fold) in various animal species [13]. There is still more controversy over the activity of AOS in the heart and liver. We found that in LR rats the AOS activity in the liver is higher than in the heart. An analogous relationship between the contents of antioxidant enzymes in the liver and heart was demonstrated by others [5,12,13]. However, other data on the relationship between the AOS components in different organs showed that the antioxidant activity of lipids [2] and the contents of water-soluble antioxidants and nonprotein thiols [10] are higher in the heart than in the liver or that there is no difference between the activities of a number of antioxidant enzymes in these organs. The latter finding agrees with our results: the AOS parameters in the studied organs are almost the same in HR rats. Consequently, the contradictions in the published data may have to do with the fact that the LPO and AOS

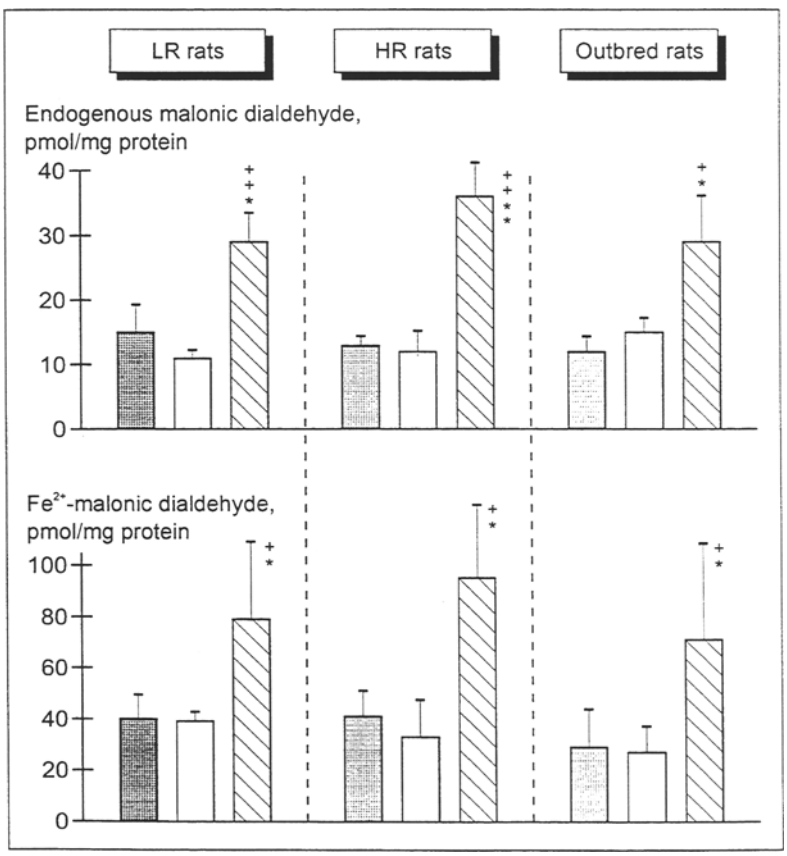

Fig. 2. TBA-reactive products in rat heart, liver, and brain.

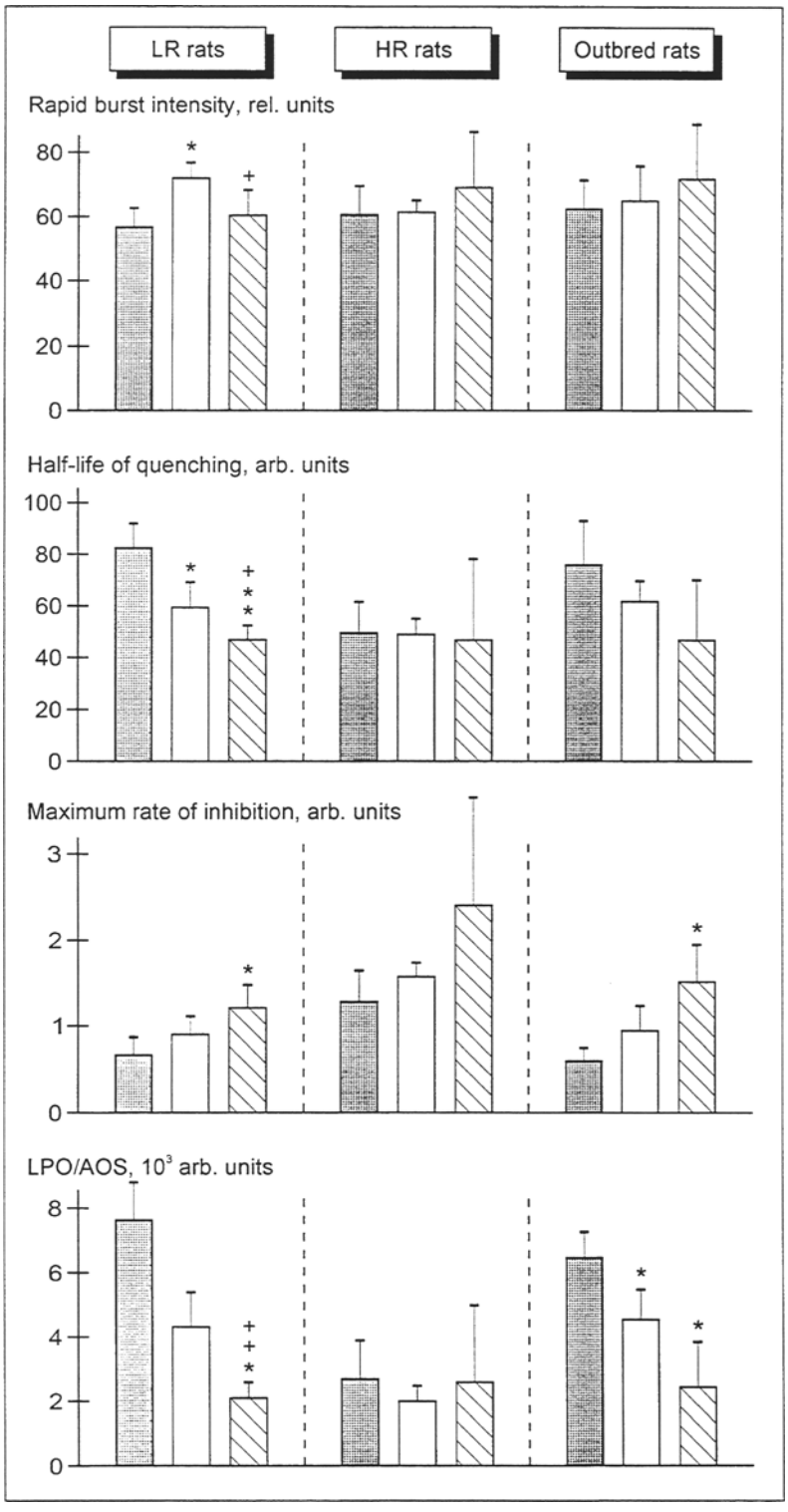

Fig. 3. Chemiluminescence in rat heart, liver, and brain.

parameters were assessed without considering the animals' resistance to hypoxia.

It should be stressed that the differences among the organs in the content of LPO products and the rate of ascorbate-induced LPO are only weakly related to the resistance of rats to hypoxia and to population peculiarities, being the same in all groups of rats. But, in contrast to LPO parameters, AOS parameters and LPO/AOS are coupled to the resistance to hypoxia: differences between these parameters in different organs are pronounced in LR rats and absent in HR rats. In outbred rats, the differences in the relationship between LPO and AOS parameters in different organs are similar to those in LR Wistar rats.

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The Microcirculatory Bed of Hamster Cheek Pouches after Occlusion of the Common Carotid Arteries

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The responses of hamster cheek pouch microvessels after occlusion of the common carotid artery on the ipsilateral side are compared. It is found that under conditions of limited inflow the microvessels preserve the inflow on the left side and the outflow on the right side, with venular constriction predominating in the left cheek and arteriolar in the right.

Key Words: *microvessels; morphometry; ischemia; asymmetry*

The state of the microcirculation in organs remote from the brain under conditions of limited blood flow to the latter has been discussed repeatedly [2]. The differences in the microcirculatory bed (MB) of paired structures have been studied. Morphofunctional asymmetry has been established for pial vessels in symmetrical areas of the feline brain [9], microvessels of rabbit ears, hamster cheek pouches, and symmetrical fragments of frog lingual vessels [3-6]. Hemodynamic characteristics of the symmetrical common carotid arteries have been studied and their functional lability has been demonstrated [7,8]. In view of the fact that blood is supplied to hamster cheeks from the common carotid arteries, it can be hypothesized that restriction of the blood flow to the brain will be reflected in the microcirculation of the cheeks. In the present study we analyzed microcirculatory disorders in hamster cheeks after occlusion of the common carotid artery on the ipsilateral side.

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MATERIALS AND METHODS

Experiments were performed on 10 hamsters (140-180 g) under Nembutal anesthesia (0.09 mg/g body weight). Biomicroscopy of the vascular bed was performed by the method of transparent chambers [10]. The field of view was illuminated via an optical fiber

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