Free Radical Processes in the Liver of Adult and Old Rats during Stress

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We measured the content of free radical lipid peroxidation products, intensity of induced lipid peroxidation, and concentration of carbonylated proteins in the liver of adult (10-12 months) and old rats (22-25 months) subjected to 30-min immobilization. Changes in the intensity of induced lipid peroxidation in homogenates and accumulation of carbonylated proteins in subcellular liver fractions indicate that immobilization stimulates free radical generation in the liver of both adult and old rats. The intensity of this process in old rats surpassed that in adult animals.

Key Words: *oxidative stress; immobilization; aging; liver*

Stress-induced changes in the intensity of free radical processes in the liver during late ontogeny remain little studied [3,6,9]. Study of this problem would allow us to determine the mechanisms of age-related impairment of the resistance to stress and develop new approaches for the correction of this disorder. Here we studied changes in free radical oxidation (FRO) of lipids and proteins in the liver of adult and old rats during immobilization stress.

MATERIALS AND METHODS

Experiments were performed on 60 adult (10-12 months) and old Wistar rats (22-25 months). Some animals were intact. Other rats were subjected to immobilization stress (fixation on the back for 30 min). The efficiency of stress was evaluated by blood concentrations of 11-hydroxycorticosteroids and catecholamines.

The animals were decapitated. Liver samples were frozen in liquid nitrogen and used to measure the contents of conjugated dienes (CD) [12], Schiff bases [13], carbonylated proteins [1], and thiobarbituric acid-reactive (TBA-reactive) substances [10]. In a special series the amount of carbonylated proteins was measured in mitochondrial, microsomal, and cytosolic fractions of the liver.

For evaluation of the intensity of induced lipid peroxidation (LPO) the liver was washed from blood, minced, and homogenized in 0.1 M phosphate buffer (pH 7.4). Freshly prepared homogenates (10%) containing 2-4 mg protein were transferred to the reaction mixture consisting of 0.1 M Tris-chloride buffer (pH 7.4), 0.8 mM ascorbic acid, and 1.2 μM More salt and incubated at 37°C. CD concentration was measured in aliquots taken at fixed time intervals.

Protein content was determined by the method of Lowry. The results were analyzed by Student's *t* test.

RESULTS

The concentration of carbonylated proteins in the liver of old rats was 33% lower than in adult animals (Table 1). Similar differences were revealed in subcellular fractions. In old rats the amount of carbonylated proteins in mitochondrial, microsomal, and cytosolic fractions was lower than in adult animals by 33, 43, and 29%, respectively (Fig. 1). The intensity of induced LPO decreased, and the dynamics of this process was different in liver homogenates from old rats (Fig. 2).

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TABLE 1. Content of LPO Products in Rat Liver after Immobilization Stress (*M*±*m*)

Index	Adult rats		Old rats	
	intact	stress	intact	stress
Conjugated dienes				
nmol/g lipids	56±19 (4)	51±2 (6)	50±1 (6)	25±2* (6)
nmol/g	23.8±0.5 (6)	22.7±1.4 (6)	22.5±0.3 (6)	18.0±0.6 (6)
TBA-reactive substances, μmol MDA/g	1.4±0.1 (5)	1.3±0.1 (6)	1.6±0.1 (6)	1.2±0.1* (6)
Schiff bases, U/g	46±12 (5)	74±12 (6)	31±6 (6)	28±5 (6)

Note. Number of animals is shown in parentheses. *$p<0.05$ compared to intact rats.

Oxidative stress develops in tissue of internal organs during late ontogeny [5]. However, we did not reveal these changes in the liver. Probably, high resistance to oxidative stress is one of the factors that determine the ability of this organ to retain functional activity during late ontogeny [14]. Age-related changes in the resistance of hepatocytes to prooxidants and alteration of enzyme systems utilizing FRO products play an important role in this phenomenon.

Immobilization was accompanied by changes in the content of products of FRO of lipids and proteins in the liver, which depended on the age of rats. After immobilization the concentrations of CD and TBA-reactive substances in old animals decreased by 51 and 25%, respectively, compared to the baseline level (Table 1). By contrast, immobilization had no effect on the amount of CD, TBA-reactive substances, and Schiff bases in the liver of adult rats.

Immobilization stress had no effect on the content of carbonylated proteins in the liver in both age groups and this parameter did not differ from that in intact rats. It should be emphasized that in adult rats the concentration of these metabolites after immobilization increased in mitochondrial and microsomal fractions (by 25 and 40%, respectively), but decreased in the cytosolic fraction (by 29%, Fig. 1). In old rats subjected to immobilization stress the content of carbonylated proteins increased in mitochondria and microsomes (by 75 and 150%, respectively), but remained unchanged in the cytosol.

Accumulation of carbonylated proteins in mitochondrial and microsomal fractions suggests that immobilization promoted oxidative stress in hepatocytes in both age groups. The degree of oxidative stress in old rats was higher than in adult animals (Figs. 1 and 2). It was probably related to age-related decrease in

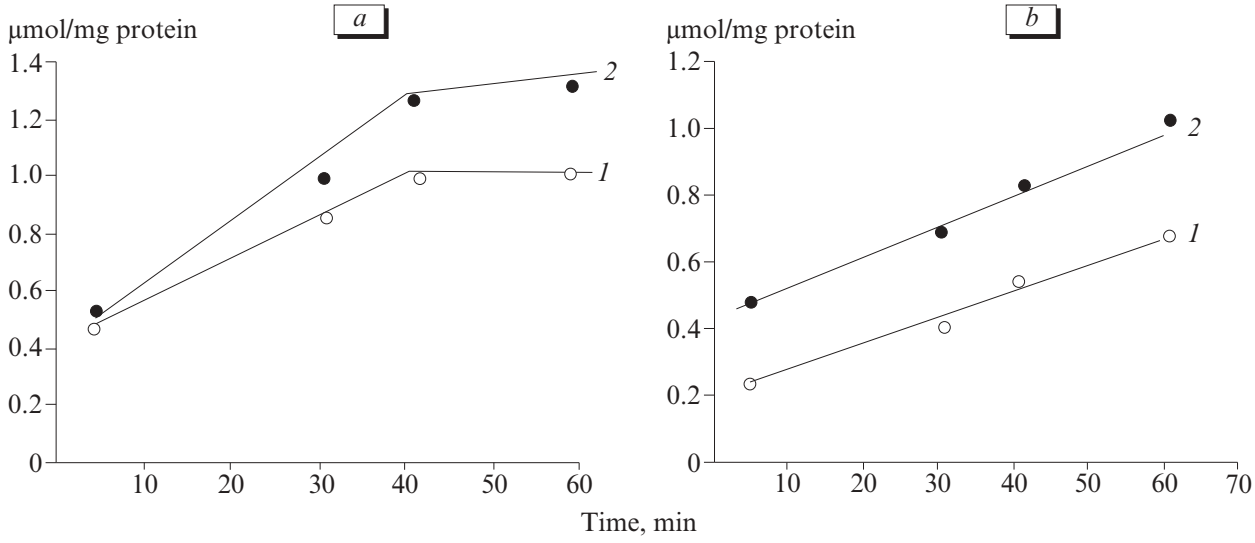

Fig. 1. Accumulation of conjugated dienes (μmol/mg protein) during induced LPO in liver homogenates from adult (*a*) and old rats (*b*): intact (*1*) and stressed animals (*2*). Here and in Fig. 2: mean of 5-6 measurements.

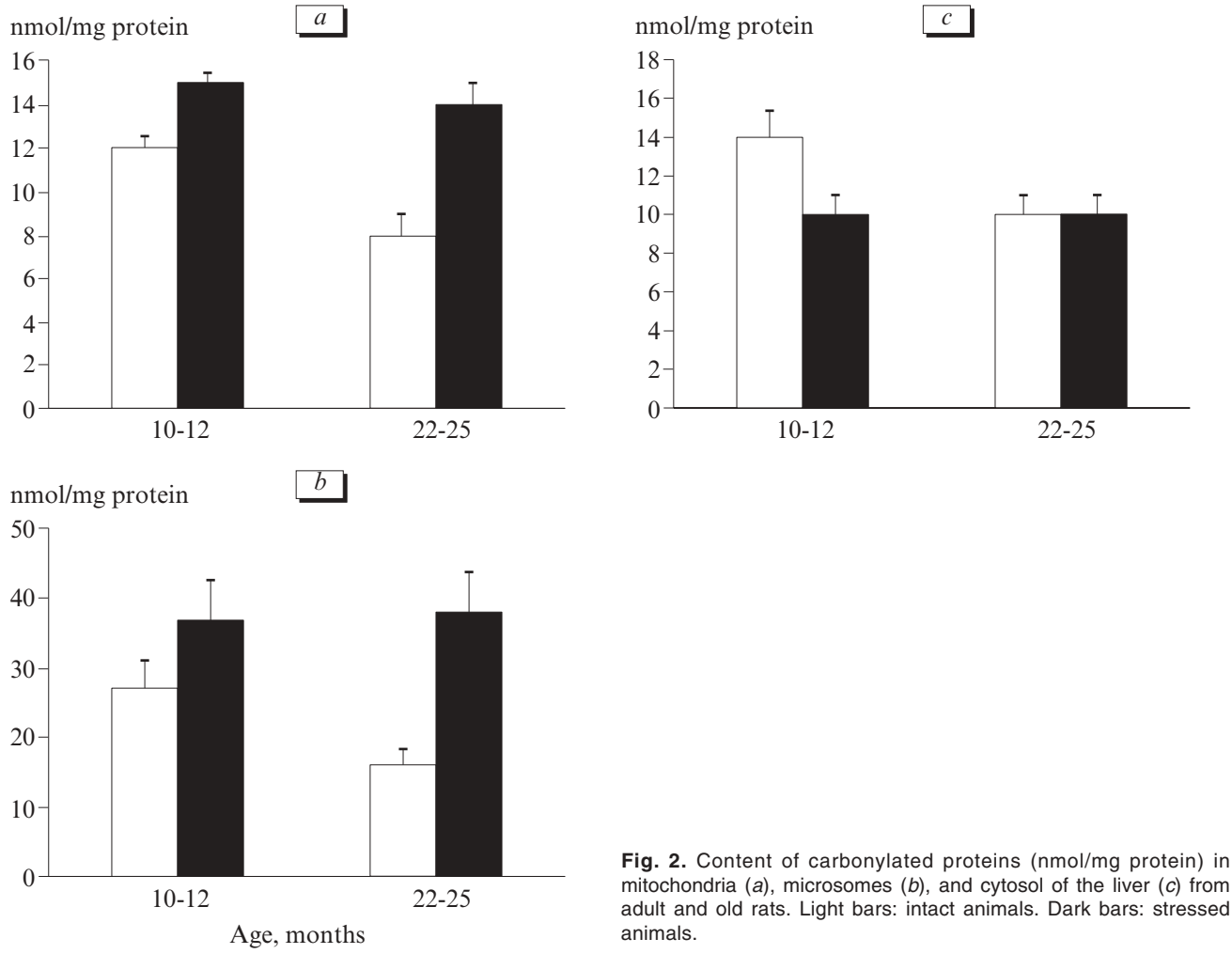

Fig. 2. Content of carbonylated proteins (nmol/mg protein) in mitochondria (*a*), microsomes (*b*), and cytosol of the liver (*c*) from adult and old rats. Light bars: intact animals. Dark bars: stressed animals.

activity of the antioxidant system [2]. These changes accompany dysfunction in membrane electron-transport chains during aging and stimulate radical generation in mitochondria and microsomes [3,11]. This promotes generation of reactive oxygen species and leads to local accumulation of carbonylated proteins.

Our findings suggest that aging is associated with increased risk of oxidative stress in the liver of rats during immobilization. Age-related variations in functional activity of membrane redox systems [11] and changes in catalytic properties of first-level antioxidant enzymes play an important role in this process.

Stimulation of FRO probably serves as a nonspecific factor that increases organism's resistance to immobilization stress [4]. However, the efficiency of this process markedly decreases during accumulation of cytotoxic FRO products in the cytosol. Compensatory stimulation of enzymes degrading products of FRO of lipids and proteins contributes to adaptation of cells in internal organs to stress [7,8].

Our results suggest that functional activity of metabolic systems utilizing products of protein FRO in the liver is high in adult and old rats. It decreases the negative effect of FRO products on hepatocytes in animals of both age groups. These changes reduce an adaptive role of oxidative stress in the liver during immobilization stress. Stimulation of FRO in liver cells of old rats exposed to immobilization probably occurs against the background of reduced antioxidant capacity. Therefore, the risk that oxidative stress would produce a negative effect on hepatocytes markedly increases in old rats. The state of "strain" in mechanisms underlying adaptation of the liver to adverse environmental factors contributes to a decrease in organism's resistance to stress during aging.

REFERENCES

1. E. E. Dubinin, *Vopr. Med. Khim.*, No. 4, 36-47 (2000).
2. E. B. Men'shikova, N. K. Zenkov, and S. M. Shergin, *Biochemistry of Oxidative Stress* [in Russian], Novosibirsk (1994).
3. G. I. Paramonova, *Dokl. Akad. Nauk Ukrainy*, No. 6, 174-177 (1994).
4. R. G. Allen and M. Tresini, *Free Radic. Biol. Med.*, **28**, No. 3, 463-499 (2000).

5. M. Barbieri, M. R. Rizzo, D. Manzella, *et al*., *Exp. Gerontol*., **38**, Nos. 1-2, 137-143 (2003).
6. J. Bejma and L. L. Ramires Ji, *Acta Physiol. Scand*., **169**, No. 4, 343-351 (2000).
7. R. Dunlop and K. Rodgers, *Free Radic. Biol. Med*., **33**, No. 7, 894-899 (2002).
8. H. Esterbauer and H. Zollner, *Biochem. J.*, **28**, No. 2, 363-373 (1985).
9. J. Faff and A. Frankiewich-Jozko, *Eur. J. Appl. Physiol. Occup. Physiol*., **75**, No. 5, 413-417 (1997).
10. G. Muller, A. Fruhant, and B. Z. Mathias, *Gzamte. Um. Med. Grenzgeb*., **41**, No. 3, 673-676 (1986).
11. H. Nohl, *Age*, **13**, No. 1, 104-105 (1990).
12. R. O. Recknagel and A. K. Choshal, *Nature*, **210**, 1162-1163 (1966).
13. C. A. Rice-Evans, A. T. Diplock, and M. C. R. Symons, *Laboratory Techniques in Biochemistry: Techniques in Free Radical Research*, London (1991).
14. D. L. Schmucker, *J. Gerontol*., **53**, No. 5, 15-20 (1998).