EFFECT OF CHRONIC EMOTIONAL STRESS ON THE STATE OF LIPID PEROXIDATION IN TISSUE AND BLOOD OF EMOTIONAL AND UNEMOTIONAL RATS

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UDC 612.397.2-06:612.821.3]-06.613.863

KEY WORDS: stress, lipid peroxidation; tissue; blood.

In view of data showing that the intensity of behavioral and somatic pathological disturbances induced by stress depends on the type of the animals' emotional-behavioral reactivity [1], of population differences in the distribution of lipid and lipoprotein content in man [7], and also on ideas regarding instability of the various components of the system inhibiting free-radical oxidation (FRO) [3], it was decided to study the state of lipid peroxidation (LPO) at tissue and functional system levels in emotional and unemotional rats (ER and UR, respectively) before and after exposure to chronic emotional stress.

EXPERIMENTAL METHOD

ER and UR (8 males weighing 250-300 g in each group), previously selected (from a noninbred population) on the basis of behavioral features [2], were subjected to chronic (for 7 days) stress by behavioral deprivation of fast sleep by the small platform method [12]. Behavioral changes were recorded 1 h after the end of stress. Four intact animals from the groups described above were used as the control. The intact and experimental rats (2 h after stress) were decapitated. The peroxide resistance of the erythrocytes [11] was determined in the blood, and concentrations of lipoproteins [6] and their hydroperoxides [4] in the serum. The content of LPO products was determined in the brain, liver, and heart, which were removed quickly in the cold. To determine the rate of spontaneous LPO not induced by pro-oxidants, samples of tissue homogenates, made up in 0.025 M Tris-HCl buffer, pH 7.4, containing 0.175 M KCl, were incubated on a water bath at 37°C for 60, 120, and 180 min with continuous shaking. The degree of peroxide formation was judged from the accumulation of malonic dialdehyde (MDA) in the reaction with 0.8% solution of 2-thiobarbituric acid (TBA) [9]. Protein was precipitated with TCA (final concentration 5%) followed by centrifugation for 10 min at 4000g. The content of lipid peroxides was expressed in nanomoles MDA per milligram of protein, determined by Lowry's method. Somatic reactions were assessed by measuring the area of ulceration of the stomach, and changes in body weight and the weight of the adrenals and thymus. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of evaluation of the behavioral and somatic manifestations in UR and ER after exposure to stress were identical with those obtained during previous investigations [1]. According to such parameters as effective reactions to test stimuli, a twofold increase in weight of the adrenals, considerable atrophy of the thymus and a reduction in body weight, extensive ulceration of the gastric mucosa, and injection of the vessels of the internal organs, ER had a greater tendency than UR to develop pathological reactions. Differences in the level of resistance of the animals to stress were accompanied by different rates of accumulation of LPO products in the tissue before and after exposure to stress (Table 1).

It will be clear from Tables 1 and 2 that ER were distinguished by a high blood level of lipoprotein acylhydroperoxides and by a high rate of MDA accumulation in the tissues. The absence of differences in values for peroxide hemolysis (an indicator of total tocopherol provision; see Table 2) explains the high level of FRO in the brain, liver, and myocardium of ER

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Medical Stomatologic Institute, Poltava. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 12-14, July, 1985. Original article submitted October 4, 1984.

 0007-4888/85/0007-0848$09.50

TABLE 1. Changes in Concentration of TBA-Active Products (in nanomoles/mg protein) in Tissues of ER and UR after Chronic Stress

Experimental conditions	Group of animals	Brain			Liver			Heart		
		incubation time, min								
		60	120	180	60	120	180	60	120	180
Before stress (control)	1. UR	0,22±0,01	0,39±0,05	0,47±0,03	0,14±0,03	0,18±0,02	0,27±0,08	0,03±0,01	0,22±0,06	0,27±0,12
	2. ER	0,31±0,05	0,45±0,10	0,54±0,02	0,19±0,05	0,31±0,03	0,50±0,05	0,06±0,02	0,18±0,09	0,40±0,15
	P_{1-2}	<0,05	—	<0,05	—	<0,001	<0,001	—	—	<0,001
After stress	3. UR	1,85±0,03	2,51±0,20	3,13±0,03	0,46±0,07	0,84±0,10	1,04±0,11	0,02±0,01	0,24±0,10	0,14±0,01
	P_{2-3}	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	—	—	<0,05
	4. ER	3,03±0,22	4,82±0,09	2,78±0,17	0,91±0,12	1,13±0,08	0,97±0,03	0,12±0,03	0,36±0,08	0,92±0,06
	P_{2-4}	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,05	<0,001	<0,001
	P_{3-4}	<0,001	<0,001	—	<0,001	<0,001	—	<0,001	—	<0,001

TABLE 2. Changes in Blood Levels of Total β- and Pre-β-Lipoprotein Fraction in ER and UR after Chronic Stress

Experimental conditions	Group of animals	β- and pre-β-lipoproteins, g/liter	Hydroperoxides of β- and pre-β-lipoproteins, extinction units/ml serum	Peroxide hemolysis of erythrocytes, per cent
Before stress (control)	1. UR	1,34±0,11	0,795±0,055	7,6±1,6
	2. ER	1,72±0,18	1,005±0,077	7,2±1,1
		P_{1-2}<0,001	P_{1-2}<0,001	—
After stress	3. UR	0,63±0,12	0,765±0,072	13,4±2,3
		P_{1-3}<0,001		P_{1-3}<0,05
				P_{3-4}<0,05
	4. ER	1,02±0,15	1,487±0,083	23,5±2,9
		P_{2-4}<0,001	P_{2-4}<0,001	P_{2-4}<0,001
		P_{3-4}<0,05	P_{3-4}<0,001	P_{3-4}<0,001

as due to the low functional activity of their antioxidant system and the possibility of greater depletion of the antiradical inhibitor chain in these tissues.

The sharp rise in the rate of MDA accumulation induced in the rat tissues by emotional stress (expectation of falling into the water against the background of chronic hypodynamia) points to breakdown of the FRO inhibition system and the development of a peroxidation syndrome. The greatest increase (5-9-fold) in the MDA concentration was observed in brain tissue homogenates, a smaller increase in the liver (3-5-fold), and only a twofold increase in the myocardium of ER. This was due to the high phospholipid concentration in membranes of the synaptic endings, a high molecular oxygen consumption (25% of that utilized by the body), and also, perhaps, to an increased concentration of potential FRO inducers, namely free-radical forms of biogenic amines. Activity of tyrosine hydroxylase, the key enzyme of catecholamine biosynthesis, in the hypothalamus of rats of the different emotional types is increased by 3-4 times. A greater increase in activity of the enzyme is observed in ER [1]. The same trend of differences between the groups also was found with respect to the rate of MDA accumulation in brain, liver, and heart tissues. In both cases stress-induced functional loading amplified the individual differences. This could reflect individual differences in coordination between processes of LPO, synthesis and degradation of biogenic amines, and inhibition of FRO.

It can accordingly be concluded that the sharper increase in the intensity of FRO in ER during stress is due to the high reactivity of their neuroendocrine systems, together with the correspondingly higher level of the oxygen and fatty acid supply to their tissues and the relatively low functional activity of the physiological antioxidant system.

The higher lipoprotein (cholesterol carriers) level in the blood of intact ER, and also data on behavioral changes [2] indicate a predominantly emotionally-negative typological status. High blood cholesterol levels are recorded when such states are induced in animals by stimulation of the hypothalamus [10], which participates directly in the mechanism of the stress reaction. Emotional stress leads to activation of competitive FRO of fatty acids and triglycerides, which have not been utilized for a long time in the course of enzymic oxidation. Under these conditions the rate of hydroperoxide accumulation in emotional animals rose significantly even if the lipoprotein concentration was lower. This correlates distinctly with differences between hemolytic resistance of the erythrocytes in the two groups.

Increased erythrocyte hemolysis in ER and ultrastructural changes in the hypothalamic and myocardial microcirculation of these animals (separation of endothelial junctions and escape of erythrocytes into the intercellular space [4, 5]) are pathological manifestations of identical genesis.

Sixfold individual differences were observed in the accumulation of TBA-active products in the myocardium after stress, which correlate with the qualitatively different ultrastructural changes [5]. Rupture of the sarcolemma of the myocytes, with release of mitochondria and of numerous glycogen granules into the intercellular space, and also the presence of multiple conglomerates of aggregating platelets in the lumen of the vessels in emotional animals, are evidence of profound pathological changes with disturbances of coordination of lipid and carbohydrate metabolism, the leading role in whose genesis is played by LPO. Somatic pathological changes observed at the macro- and ultrastructural levels in ER are evidence of a stress-induced profound stage of exhaustion, in which the thrombus-forming properties of the blood are enhanced. This leads to activation of the humoral agent of the anticlotting system, namely lipoprotein lipase, which forms complexes with lipoproteins, potentiates their fibrinolytic activity, and may hydrolyze the triacylglycerides present in the composition of the complexes themselves [8]. These factors can significantly affect concentrations of β- and pre-β-lipoproteins in animals in a state of prethrombosis due to prolonged emotional stress.

These data showing how changes in the FRO level and tissue damage arising during stress depend on the type of emotional response are of great importance for the explanation of the increased risk of ischemic heart disease and of other diseases known to exist in highly emotional persons, those with so-called type A behavior. Meanwhile these data indicate that besides psychotropic drugs, the use of antioxidants may also be indicated for the pharmacoprophylaxis of the consequences of stress.

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CORRELATION BETWEEN CHANGES IN BLOOD RHEOLOGIC PROPERTIES AND MICROCIRCULATORY DISTURBANCES IN EARLY AND LATE STAGES OF EXPERIMENTAL HYPERLIPOPROTEINEMIA

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UDC 616.153.963'915-008.61-07:617.151.5-036.4-092.9

KEY WORDS: hyperlipoproteinemia; hemorheology; microcirculation; microvessels.

Clinical and experimental investigations have yielded evidence of the effect of hyperlipoproteinemia (HLP) on the rheologic properties of the blood and on the microcirculation (MC) [1-3, 5, 10-12]. We know that HLP is one of the leading risk factors of atheroschlerosis and its complications: ischemic heart disease (IHD) and myocardial infarction. According to some authorities changes in blood rheologic properties are similar in character whether atherosclerotic changes are present in the large arteries or not [7]. Accordingly, the study of the mechanisms of disturbance of the rheologic properties of the blood and of MC in the early stages of development of HLP, in the preclinical stage of the disease, becomes particularly important.

This paper describes a comparative study of the rheologic properties of the blood and MC in the early and late stages of experimental HLP.

EXPERIMENTAL METHOD

Twenty male Chinchilla rabbits weighing 2.3-2.8 kg were used. Experimental HLP was induced by Anichkov's method in Yushchenko's modification: 0.5 g/kg cholesterol (Ch) in 100 g of carrot. Blood for testing was taken from the auricular vein of the rabbits before Ch loading, 15 h after a single Ch loading, and also after the animals had been kept for 1, 3, 9, 30, 60, and 90 days on an atherogenic diet (AGD). Total Ch (TCh) (by the method of Girard and Assous), chylomicrone (ChM), and low (LDL), very low (VLDL), and high (HDL) density lipoproteins (LP) [8] were determined in the blood serum.

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 15-19, July, 1985. Original article submitted October 16, 1984.

