LIPID PEROXIDATION AND RETINAL INJURY IN STRESS

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Emotional-painful stress (EPS) regularly leads to generalized activation of lipid peroxidation (LPO), and this is expressed as accumulation of LPO products in various tissues: the brain, heart, muscles, etc. [5, 6]. Activation of LPO has been shown to be a key stage in the pathogenesis of stress injury to the myocardium, and LPO inhibitors can prevent both the accumulation of LPO products and the development of the complex of stress injuries to the heart [7]. Meanwhile the role of accumulation of LPO products in the brain, induced as a result of EPS, has not been studied. A convenient object with which to study this problem is the retina, which develops in mammals from brain tissue and contains high concentrations of polyene lipids, undergoing LPO with a high reaction velocity [4]. Changes in retinal function can be easily and conveniently recorded as the electroretinogram (ERG) in response to photic stimulation. Psychogenic (stressor in origin) disturbances of vision also have been the subject of research in clinical ophthalmology for a long time [1]. However, the effect of stress on metabolism and functions of the retina, the peripheral part of the visual analyzer, has not previously been investigated.

The object of this study was to examine the action of EPS on endogenous LPO reactions in the retina, on the main components of the LPO regulation system in the retina, namely superoxide dismutase and α-tocopherol, and on electrical activity of the retina.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170-200 g. EPS was reproduced in the form of a so-called anxiety neurosis [11]. Antioxidants, namely 4-methyl-2,6-di-tert-butylphenol (MDTB) and OP-6, belonging to the hydroxypyridine class, were injected intraperitoneally into the animals once daily for the 3 days before EPS (corresponding doses 100 and 120 mg/kg body weight). A single injection of inderal in a dose of 0.5 mg/kg was given to the animals before EPS.

The ERG of the rats was recorded by means of a wick electrode (active electrode) on the EEGP-4-02 encephalograph. Photic test stimulation was applied with a type FS-1 photostimulator (flash energy 0.3 J). The pupil was dilated with 1% homatropine solution and the eyes were anesthetized with 1% amethocaine. The rats were decapitated 2 h after the end of exposure to stress. Lipids were extracted from the retina [12] with a 2:1 mixture of chloroform and methanol with the addition of the antioxidant MDTB (1 mg to 100 ml of the mixture). The concentrations of primary LPO products were determined spectrophotometrically (Shimadzu MPS-50L spectrophotometer) using the characteristic absorption maximum (232 nm) for diene conjugates in the UV-spectrum of solutions of lipids in methanol—heptane (5:1) [8]. The end products of LPO — products of interaction between short-chain dialdehydes and aminophospholipids — were recorded [10] as fluorescence spectra of solutions of lipids in chloroform (wavelength of excitation 365 nm, of emission 420-450 nm on an Aminco Bowman spectrofluorometer. Before each series of measurements the instrument was calibrated against a standard solution of quinine sulfate (1 μg/ml in 0.1 N H_2SO_4). The content of α-tocopherol in the retina was

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TABLE 1. Content of Endogenous LPO Products in Rat Retina 2 h after EPS (M ± m)

Conditions	Lipid hydroperoxides (E_{232}, optical density units)	LPO end product (intensity of fluorescence, relative units)
Control	0,51±0,06 (n=11)	4,7±0,3 (n=11)
EPS	0,83±0,07 (n=11)	11,4±0,7 (n=11)
EPS + antioxidant:		
MDTB	0,52±0,03 (n=8)	5,2±0,6 (n=8)
OP-6	0,55±0,06 (n=8)	4,8±0,7 (n=8)
EPS + inderal	0,67±0,10 (n=8)	5,6±0,3 (n=8)

TABLE 2. Effect of EPS on Superoxide Dismutase Activity and α-Tocopherol Content in Rat Retina (M ± m)

Parameter studied	Control	After EPS
Superoxide dismutase activity units/mg protein	0,58±0,03 (n=10)	0,63±0,04 (n=10)
α-Tocopherol content, mg/mg protein	0,47±0,05 (n=8)	0,29±0,07 (n=8)

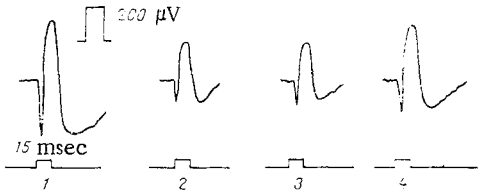

Fig. 1. Typical traces of ERG of rats in control (1) and 2, 24, and 48 h after EPS (2, 3, and 4, respectively).

determined by a modified method [13], involving the recording of characteristic fluorescence (wavelength of excitation 240 nm, of emission 340 nm). Superoxide dismutase activity in the retina was measured as described in [2].

EXPERIMENTAL RESULTS

As Table 1 shows, 2 h after EPS the content of endogenous primary (hydroperoxides of polyene lipids) and end (fluorescent Schiff bases) products of LPO in the retina was increased by 1.5 and 2.5 times, respectively. This means that in the retina, just as in other tissues studied previously [5, 6], EPS leads to activation of endogenous LPO.

One cause of the accumulation of endogenous LPO products may be the onset of disturbances in the system controlling LPO reactions, the main components of which in the retina are α-tocopherol and superoxide dismutase [2]. The results in Table 2 show that EPS caused no changes in superoxide dismutase activity in the rat retina. Meanwhile the content of α-tocopherol (vitamin E), a natural inhibitor of free-radical oxidation, in the retina was significantly reduced after EPS (by more than 35% of its initial level). Hence, as a result of exposure to EPS a deficiency of the natural antioxidant vitamin E arises in the retina. The correction of

TABLE 3. Changes in Amplitude of *a* and b Waves of ERG of Rats during EPS and Protective Action of Antioxidants (M ± m)

Experimental conditions	ERG wave	Control	Time after EPS, h			
			2	24	48	72
Control	a	267±17	109±8	120±10	130±20	260±30
	b	625±47	256±18	300±23	340±37	630±50
MDTB	a	267±17	175±17	260±19	260±18	—
	b	625±47	510±28	617±34	620±35	—
OP-6	a	267±17	180±19	253±22	257±19	—
	b	625±47	500±26	610±38	613±28	—

this deficit by means of synthetic antioxidants ought probably to prevent the accumulation of LPO products induced in the retina by EPS. In fact, preliminary administration of lipid-soluble (MDTB, 100 mg/kg) or water-soluble (OP-6 belonging to the hydroxypyridine class, 120 mg/kg) antioxidants completely abolished the effect of EPS: LPO products did not accumulate (Table 1). The question accordingly arises: Is the deficit of the natural antioxidant (a fall in its concentration) the primary mechanism of LPO activation in the retina during EPS or does it take place secondarily as a result of interaction between α-tocopherol and radical intermediates of LPO, whose appearance during exposure to EPS is attributable to other causes? The answer to this question is given by the results of experiments with the β-adrenoblocker inderal (Table 1). Inderal, which itself is not an inhibitor of LPO, whether induced by nonenzymic (Fe^{++} + ascorbate) or enzymic (Fe^{++} + NADPH) methods, caused virtually complete inhibition of LPO induced by EPS in the retina. The β-adrenoblocker inderal thus prevents LPO activation during EPS and the mechanism of its action differs in principle from that of antioxidants, for it prevents excessive activation of adrenoreceptors by catecholamines. Although a more detailed examination of the possible mechanisms of LPO activation during EPS lies outside the scope of this investigation, it may nevertheless be noted that according to data in the literature [9] autooxidation of catecholamines is accompanied by generation of superoxide anion-radicals, which can initiate LPO reactions [7].

A study of the action of EPS on electrical activity of the retina (Fig. 1) revealed a decrease in amplitude of the *a* and b waves of the ERG, recordable 2 h after EPS and continuing for as long as 20 h after stress. The writers showed previously that induction of LPO in the outer segments of photoreceptors leads to modification of the visual protein rhodopsin [3], and in the isolated retina *in vitro* it leads to inhibition of electrical activity [14]. Comparison of this fact with the results described above suggests that the mechanism of **inhibition of** retinal electrical activity during EPS is activation of endogenous LPO *in vivo*. Experimental confirmation of this hypothesis is given by the data in Table 3, which demonstrate the protective effect of antioxidants (MDTB, OP-6) administered beforehand to the animals against retinal damage induced by EPS.

As a result of EPS, LPO products causing damage to the electrical activity of the retina thus accumulate in the retina in rats. Prevention of LPO activation by antioxidants during EPS protects the retina **against damage** caused by EPS.

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HUMAN MUSCLE GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE IN ATHEROSCLEROSIS

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Changes in the functional activity and certain properties of glyceraldehyde phosphatase dehydrogenase (GAPD) from the skeletal muscles of rabbits with experimental atherosclerosis were discovered previously [2, 3].

The object of the present investigation was to develop a method of obtaining the enzyme in a pure crystalline form from the muscles of patients with atherosclerosis and to study the catalytic activity and some physicochemical properties of this enzyme.

EXPERIMENTAL METHOD

GAPD was isolated from autopsy material. Muscles were taken from 40 cadavers not more than 12-18 h after death. Death was accidental in all cases due to automobile and railroad accidents and gunshot and knife wounds. Persons dying from organic disease of the liver, kidneys, endocrine system, etc., were excluded. Material taken from subjects with no morphological signs of atherosclerosis was included in the control group.

GAPD was isolated in the crystalline form by preliminary extraction of the muscles with potassium phosphate followed by fractional precipitation with ammonium sulfate to remove ballast proteins. The method of fractionation of muscle proteins from human muscles in [1] was used as the prototype for development of the method in the present case.

EXPERIMENTAL RESULTS

Distinguishing features of GAPD in atherosclerosis came to light even at the stage of isolation of the enzyme. They were expressed, in particular as changes in the crystallization process: the earlier appearance of crystals but, at the same time, their low stability in the mother liquor, their smaller size, and a tendency to be arranged in groups. A typical feature of the enzyme from normal human muscle tissue is that it forms rod-shaped crystals with clear edges. The shapes of the GAPD crystals obtained by the present writers agree with those described in the literature [4]. The homogeneity of the preparations was confirmed by electrophoresis in polyacrylamide gel (Fig. 1). The GAPD was concentrated in the gel as a single band. Enzymes from muscles from patients with atherosclerosis migrate under similar conditions more slowly and are located nearer to the starting line.

Comparison of the specific activity of the enzymes from normal and atherosclerotic subjects showed that in the latter GAPD activity was 42% lower and amounted on average to 2.7 ± 0.2 μ-moles NADH/mg·min. GAPD from muscles of patients with atherosclerosis, when dissolved in wa-

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