COMPARATIVE QUANTITATIVE ANALYSIS OF LIPID PEROXIDATION PRODUCTS IN THE CEREBRAL CORTEX, CSF, AND PERIPHERAL BLOOD DURING EPILEPTIC ACTIVITY

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The development of various forms of epileptic activity (EA), induced by various epileptogens, in the cerebral cortex of experimental animals is accompanied by activation of lipid peroxidation (LPO) in the cortex [2, 4, 6, 8, 14]. It has been shown that an uncompensated increase in the intensity of LPO during EA can be prevented by preliminary administration of antioxidants [6-8]. Under these circumstances EA is considerably weakened and, if high doses of antioxidants are used, it may be almost completely suppressed [1, 6-8]. It has been concluded from these observations that uncompensated LPO plays a pathogenetic role in the development of EA [6, 8].

The object of this investigation was to study whether intensification of LPO during EA in the brain is accompanied by corresponding changes in the level of LPO products in the cerebrospinal fluid (CSF) and in the peripheral blood.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 150-200 g. A focus of EA was induced by application of the sodium salt of penicillin to the surface of the animals' sensomotor cortex [5]. Primary generalized EA was induced by intramuscular injection of bemegride [8]. Before the level of LPO products was determined, the rats were starved for 15-20 h.

LPO activity in the cerebral cortex was determined by measuring the concentration of primary (diene conjugates) and end (Schiff bases, products reacting with 2-thiobarbituric acid — TBA-active products) products of LPO. For this purpose brain tissue, taken from the region of hyperactivity as described previously [6], was homogenized in the cold and, from the resulting homogenate, the fraction of unpurified synaptosomes (FUS) was isolated by the method in [12]. The concentration of TBA-active products was determined in FUS suspended in medium of the following composition (in mM): NaCl 132, KCl 5, NaH_2PO_4 1.2, $CaCl_2$ 1.2, $MgCl_2$ 1.3, glucose 10, Tris-HCl 20, pH 7.4 (20°C), by the method in [3]. The level of diene conjugates and Schiff bases was determined in lipids extracted from FUS by the method in [9]. The concentration of diene conjugates was determined by the method in [10], and of Schiff's bases as in [11]. To measure the optical density of the lipids at 233 nm in a methanol—hexane mixture (5:1 by volume), the Specord UV VIS spectrophotometer (East Germany) was used, and fluorescence of a chloroform extract of lipids at 430 nm (excitation wavelength 360 nm) was measured on the Hitachi-204 spectrofluorometer (Japan).

CSF was taken in a volume of 25-80 µl by cisternal puncture with a No. 5 injection needle. Lipids were then extracted from the CSF in the presence of ionol (10^{-4} M) and the content of Schiff's bases in the extract was determined [11].

Blood (2-3 ml) was taken from the bifurcation of the inferior vena cava into a sterile syringe with 0.5 ml of heparin solution (0.75 mg in 1 ml physiological saline). The blood

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TABLE 1. Concentrations of LPO Products in FUS Isolated from Rat Cerebral Cortex in Region of Focus of EA (M ± m)

Experimental conditions	Diene conjugates	Schiff bases	TBA-active products
Control	4,80±0,14 (4)	220±7 (4)	520±62 (10)
Focus of EA	8,88±1,30*** (4)	340±18* (4)	1280±100*** (11)
Control	0,86±0,05 (4)	115±5 (4)	340±40 (6)
Primary generalized EA	1,82±0,24** (4)	160±9* (4)	1020±100* (7)

Legend. *P < 0.01, **P < 0.02, ***P < 0.05 compared with control. Here and in Table 2, number of animals given in parentheses.

TABLE 2. Concentrations of LPO Products in CSF and Peripheral Blood Plasma of Rats with EA (M ± m)

Material studied	Experimental conditions	Diene conjugates	Schiff bases
Blood	Control	—	130±5 (4)
	Focus of EA	—	290±28** (4)
	Control	1,06±0,15 (6)	100±14 (6)
	Primary generalized EA	2,97±0,35** (7)	350±32* (7)
CSF	Control	—	100±12 (16)
	Primary generalized EA	—	225±56*** (16)

Legend. *P < 0.001, **P < 0.01, ***P < 0.1 compared with control.

thus obtained was centrifuged at 3000 rpm for 15 min on the TSLU-1 centrifuge at 0-4°C and the plasma was carefully separated, and used to extract lipids and for determination of the level of diene conjugates and Schiff bases in the extract.

The concentrations of TBA-active products were expressed in picomoles/mg protein, those of diene conjugates in micromoles/mg total lipids. The protein concentration was determined by Lowry's method [13]. The concentration of total lipids was determined by using the kit of standard reagents from Lachema (Czechoslovakia). The essence of the method is that, after hydrolysis with sulfuric acid, the lipids interact with phosphovanillin reagent with the formation of a colored complex. The concentration of Schiff bases in FUS from cerebral cortex and in blood plasma was expressed in percentages of a standard, reduced for the level of total lipids (to 1 mg). A solution of quinine sulfate (1 mg of the substance in 1 ml of 0.1 N H_2SO_4) was used as the standard, the intensity of which was taken as 100. The content of Schiff bases in the CSF was expressed as a percentage of the control (CSF of rats receiving physiological saline instead of bemegride), reduced to 1 µl of CSF taken.

EXPERIMENTAL RESULTS

It was shown previously that the development of a focus of penicillin-induced or primary generalized EA in the rat cerebral cortex is accompanied by a sharp increase in FUS, isolated from the cerebral cortex (region of hyperactivity), and in the level of TBA-active products [6, 8]. Meanwhile the results are evidence of a relatively low level of TBA-active products in FUS and in the blood plasma, and for that reason it seemed inadvisable to use this parameter to assess the state of LPO in such systems. Accordingly, the level of other LPO products — diene conjugates and Schiff bases — was measured in the CSF and blood plasma. It was shown first that during development of EA, besides an increase in the content of TBA-active products in FUS isolated from a region of EA, an increase also is observed in the level of diene conjugates and Schiff bases in FUS (Table 1).

Investigation of the CSF showed that the development of primary generalized bemegride-induced EA in rats is accompanied by a tendency for the concentration of Schiff bases in the CSF to rise (Table 2). The high value of the standard deviation for the value of n taken (n = 16) was due to the small volume of the CSF samples obtained (25-80 µl) and the possible presence of small quantities of blood in them.

The development of EA caused a sharp increase, about the same as in the cerebral cortex, in the concentration of diene conjugates and Schiff bases in peripheral blood plasma (Table 2).

Elevation of the level of LPO products was observed in both focal and primary generalized EA (Table 2). Both in blood and brain tissue the level of LPO products in the rats used as controls in the experiments with primary generalized EA, it will be noted, was about half that in rats used as controls in the experiments with focal EA (Tables 1 and 2). The reason is evidently the degree of trauma associated with the operation (exposure of the cerebral cortex, insertion of electrodes) preceding penicillin application. For induction of focal EA both the control and the experimental animals underwent the operation.

The results thus indicate that during the development of EA in rats, besides activation of LPO in the CNS, elevation of levels of LPO products in the peripheral blood also is observed.

It is not yet possible to answer the question whether the increase in the peripheral blood level of LPO during epileptic activity is a reflection of activation of peroxidation in the CNS or whether it is the result of intensification of LPO in other tissues of the body. Meanwhile, the results are evidence of the value of studying the concentration of LPO products in the peripheral blood of epileptics. It enables crisis situations to be predicted, it enables the desirability of supplementing antiepileptic therapy with substances with high antioxidative activity to be determined, and their administration monitored.

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