BLOOD ANTIOXIDANT ENZYMES DURING EPILEPTIC ACTIVITY

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An important stage in the pathogenesis of various forms of epilepsy consists of disturbances of regulation of lipid peroxidation (LPO) in the CNS [1, 3, 5, 7, 8]. When studying the mechanism of these disturbances we postulated that one cause of uncompensated activation of LPO may be insufficiency of the antioxidant system [9]. However, it was found that the acute development of bemegride-induced epileptic activity (EpA) in rats is not accompanied by decreased activity of the principal enzymes of antioxidant protection in the animals' brain tissue: superoxide dismutase (SOD), glutathione peroxidase (GP), and gluthathione reductase (GR) [9]. It is also possible that the changes sought in activity of the antioxidant enzymes developing during acute convulsions are manifested first in another, much more labile, tissue, namely blood. Finally, as was observed previously [9], a complete explanation of the role of the antioxidant system in the pathogenesis of EpA requires investigations in the course of chronic EpA.

For the reasons given above it was decided to study activity of SOD, GP, and GR in the blood of rats during development of bemegride-induced EpA, and also in the blood of epileptics with a long duration of the disease and a high frequency of fits.

EXPERIMENTAL METHOD

Experiments on acute experimental EpA were carried out on male Wistar rats weighing 180-230 g, kept on a standard diet under normal animal house conditions. Primary generalized EpA was induced by intramuscular injection of 0.5% bemegride solution in a dose of 20 mg/kg. Control animals received an injection of the same volume of physiological saline. The rats were decapitated 20 min after injection of the convulsant, i.e., 13-15 min after an attack of generalized convulsions. Blood was taken into a glass flask containing 3.8% sodium citrate solution, made up in physiological saline. The ratio of the volume of blood collected to that of the anticoagulant solution was 5:1 by volume. The blood was centrifugued in the cold (1000g) and the plasma separated. Erythrocytes were washed 3 times with cold physiological saline and lysed in the samples to determine SOD activity, as described in [12]. SOD activity in lysed rat and human erythrocytes was determined by the method in [11] at 30°C. The unit of SOD activity was taken to be the quantity of enzyme required to inhibit reduction of nitro-BT into formazan by 50% under the conditions used. GP activity in lysed human and rat erythrocytes was determined by measuring oxidation of NADPH in a coupled glutathione reductase system, using *tert*-butyl hydroperoxide [6] as the substrate at 30°C. GR activity was determined by the method in [15].

The test object with chronic EpA consisted of 13 epileptic patients (with generalized convulsions) with a duration of the disease of 7-18 years and with a frequency of fits ranging from once daily to 1-16 times a month. All the patients were men aged 23-39 years, receiving traditional anticonvulsant therapy.

Blood was taken from the cubital vein in the morning before breakfast. Plasma was obtained from the blood as described previously [5] and EDTA was added to it up to a final con-

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TABLE 1. Activity of Antioxidant Enzymes in Blood of Rats with Experimental EpA (M ± m)

Enzyme	Control	EpA
SOD, c.u./mg protein	163,5±12,1 (8)	135,4±14.0** (8)
SOD, c.u./mg hemoglobin	2,75±0,19 (8)	1,92±0,2* (8)
GP, nmoles NADPH/min/mg hemoglobin	308,5±26,9 (8)	327,5±11,2 (8)
GR, nmoles NADPH/min/mg hemoglobin	8,2±0,5 (8)	7,4±0,6 (8)

Legend. Number of animals shown in parentheses; c.u. — conventional units. *p < 0.01, **p < 0.05 compared with control.

centration of 5 mM. The samples thus obtained were kept at −20°C for 2-7 days. The α-tocopherol (TP) concentration [13], the level of free fatty acids (FFA; by the reaction of 1,5-diphenylcarbazide with an extract of copper salts of FFA [10]), the concentration of total lipids [16], and the protein concentration by Lowry's method), were determined in the blood plasma. The erythrocytes were lysed with 0.1% Triton X-100 solution. Enzyme activity in the lysate of the patients' erythrocytes was determined as described above. The hemoglobin concentration in the samples was measured by the method of Drabkin et al.

Blood from 10 volunteer blood donors, with no mental or chronic physical diseases, was used as the control. All the donors were men aged 23-37 years.

Optical density was measured on a Hitachi-320 spectrophotometer (Japan). The intensity of fluorescence was measured on a Hitachi-204 spectrofluorometer.

The following reagents were used: EDTA, xanthine, xanthine oxidase, nitro-blue tetrazolium, TP, stearic acid, and glutathione (from Serva, USA), NADPH (from Reanal, Hungary), Triton X-100 (from Ferak, Berlin); other reagents were of the chemically pure grade.

EXPERIMENTAL RESULTS

Injection of bemegride into rats in a dose of 20 mg/kg caused the development of clonicotonic convulsions in the animals after 5-7 min, so that they fell on the side and exhibited a phase of marked tonic extension. It was shown previously that under these conditions the blood level of LPO products is increased threefold in rats 20 min after injection of the convulsant, i.e., 13-15 min after a fit [4], and that the peroxide resistance of the erythrocytes is appreciably reduced [9]. In the present investigation SOD activity in peripheral blood erythrocytes was found to be reduced 20 min after injection of bemegride into the rats, but GP and GR activity remained unchanged (Table 1). The difference for SOD was significant when its activity was expressed in two ways: relative to erythrocyte protein after precipitation of hemoglobin and to their hemoglobin content.

Investigation of blood from the epileptic patients showed that the plasma levels of total protein, total lipids, and TP were unchanged compared with the control (Table 2). No differences were observed in the TP level irrespective of the method of calculation: per ml blood plasma or per mg total lipids. The plasma FFA concentration of the epileptics was higher than normal, in agreement with previous results evidence of a disturbance of regulation of LPO in these patients, toward intensification [5].

SOD and GP activity in the blood of the epileptics was 30% lower than in healthy blood donors, but GR activity was normal (Table 2). Incidentally, the difference between SOD activity in the erythrocytes of the epileptics and healthy subjects, just as in the case of SOD activity in experimental EpA, was significant when enzyme activity was expressed relative to erythrocyte protein after precipitation of the hemoglobin and also when activity was expressed relative to the hemoglobin content of the erythrocytes.

TABLE 2. Activity of Antioxidant Enzymes in Blood of Epileptic Patients (M ± m)

Parameter	Control group (blood donors)	Epileptic patients
Total plasma protein, mg/ml	78,0±2,8 (10)	81,0±4,3 (13)
Total plasma lipids, mg/ml	4,34±0,33 (10)	3,72±0,24 (13)
Plasma TP, mg/ml	10,6±0,76 (10)	10,14±0,68 (13)
Plasma TP, mg/mg protein	2,5±0,29 (10)	2,77±0,17 (13)
Plasma FFA, μg/mg lipids	86,6±7,08 (8)	121,1±19,2** (11)
SOD, c.u./mg protein	184,4±6,4 (10)	143,4±3,6** (13)
SOD, c.u./mg hemoglobin	3,2±0,1 (10)	2,52±0,06* (13)
GP, nmoles NADPH/min/mg hemoglobin	10,7±0,42 (10)	8,14±0,60* (13)
GR, nmoles NADPH/min/mg hemoglobin	8,8±0,4 (10)	8,4±0,4 (13)

Legend. Number of patients tested shown in parentheses; c.u. — conventional units. *p < 0.01, **p < 0.05 compared with control.

The development of acute experimental generalized EpA in rats was thus accompanied by a fall in the blood level of activity of SOD — an enzyme of the antioxidant system of the body catalyzing the reaction of dismutation of one of the most active forms of oxygen, namely the superoxide anion-radical ($O_2^{\cdot}$). As a result of the decrease in SOD activity, LPO initiation reactions must be activated and the products of this process must accumulate, as was observed in the blood of the experimental rats [4]. The causes of the decrease in blood SOD activity during EpA are not yet clear and, in particular, they could be inactivation of part of the enzyme pool due to interaction with substances formed or accumulating during convulsions, or the transport of some of the SOD molecules from the blood into the tissue with the greatest need for the antioxidant enzyme during EpA in order to compensate disturbances in LPO reactions, i.e., into nerve tissue.

It is perhaps this second mechanism which enables SOD activity in the animal's brain to be maintained virtually unchanged during the development of acute EpA, despite intensification of processes of radical formation in the CNS. Compensation of lipid peroxidation under these circumstances is incomplete, for the level of LPO products in the cerebral cortex of the rats and in the cerebrospinal fluid 13-15 min after the convulsion was 2 to 3 times higher than normal [5] Kagan and co-workers [2] similarly observed a threefold increase in the concentration of LPO products in the rat brain whereas SOD activity was unchanged in the case of epileptiform convulsions induced by hyperoxia. In all probability, during the development of acute EpA functional SOD insufficiency develops in nerve tissue. Evidence of this is given by the experiments of Willmore et al. [14] who showed that 5 and 15 min after injection of $FeCl_3$ solution, which induces the development of EpA, into the rat cerebral cortex, an increase in the $O_2^{\cdot}$ concentration in the cortex is recorded.

The results of the animal experiments (Table 1) are in good agreement with those of clinical investigations of epileptic patients (Table 2). The long duration of the disease and the high frequency of epileptic fits caused greater changes in activity of enzymes of the antioxidant system. For instance, besides a significant fall of SOD activity in the blood, a fall of GP activity was found in the erythrocytes.

The absence of changes affecting other components of the antioxidant system (GP activity, TP concentration) in the blood of the epileptic patients is in agreement with results obtained in the present experiments (Table 1) and previously [9]. Since the intensity of LPO is considerably increased in patients with generalized forms of epilepsy [4], maintenance of the "activity" of components of the antioxidant system at a level corresponding to the normal course of LPO evidently signifies their functional insufficiency for the new level of LPO. That this conclusion is correct is confirmed by the positive therapeutic effect of administration of TP to epileptic patients with disturbed LPO regulation [3].

This combined clinical and experimental study thus showed that the development of EpA in animals and man is accompanied by a fall in activity of antioxidant enzymes in the blood. This fall is evidence that disturbances of LPO regulation during epileptogenesis are linked, to a certain degree, with insufficiency of the antioxidant system, and in particular, with insuffiency of the antioxidant system and, in particular, with insufficiency of the enzymes composing it.

LITERATURE CITED

1. V. A. Voronko, E. V. Nikushkin, I. G. Pronina, et al., Current Problems in Disease and Recovery [in Russian], Moscow (1983), p. 72.
2. V. E. Kagan, R. N. Kopaladze, L. L. Prilipko, et al., Byull, Éksp. Biol. Med., No. 12, 16 (1983).
3. V. M. Kovalenko, G. N. Kryzhanovskii, V. S. Kovalenko, et al., Zh. Nevropatol. Psikhiat., No. 6, 892 (1984).
4. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, et al., Zh. Nevropatol. Psikhiat., No. 6, 806 (1984).
5. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, et al., Byull, Éksp. Biol. Med., No. 11, 36 (1983).
6. V. Z. Lankin, A. K. Tikhadze, A. A. Kovalevskaya, et al., Dokl. Akad. Nauk SSSR, 261, No. 6, 1467 (1981).
7. V. G. Mkhitaryan, A. A. Megrabyan, G. E. Badalyan, et al., Zh. Éksp. Klin. Med., No. 3, 237 (1983).
8. E. V. Nikushkin, V. E. Braslavskii, and G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiat., No. 6, 810 (1981).
9. I. R. Typeev, M. M. Bordyukov, G. N. Kryzhanovskii, et al., Byull. Éksp. Biol. Med., No. 11, 538 (1985).
10. V. A. Shaternikov and L. A. Savchuk, Lab. Delo, No. 10, 598 (1964).
11. C. Beauchamp and I. Fridovich, Analyt. Biochem., 44, 276 (1971).
12. J. Maral, K. Puget, and A. M. Michelson, Biochem. Biophys. Res. Commun., 77, 1525 (1977).
13. S. L. Taylor, M. H. Lamden, and A. L. Tappel, Lipids, 11, 530 (1976).
14. L. J. Willmore, M. Hiramatsa, H. Kochi, et al., Brian Res., 277, 393 (1983).
15. Y. Yoshihito and R. Konichi, Blood, 43, 99 (1974).
16. Z. Zöllner and K. Kirsch, Z. Ges. Exp. Med., 135, 545 (1962).

EFFECT OF LIPID COMPOSITION OF LIPOSOMES ON THEIR CLEARANCE FROM THE BLOOD STREAM AND ACCUMULATION IN THE MOUSE LIVER

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Incorporation of drugs inside lipid vesicles (liposomes) has a marked effect on their pharmacodynamic and therapeutic properties. By the use of liposomes it is possible to control the circulation time of a drug or its selective accumulation in particular organs. Targeted transport of liposomes to particular tissues of cells is a fundamental task in the use of liposomes as containers for drug transport in the body. To accomplish this task, liposomes must satisfy at least two demands: they must have access to the target cells and must bind preferentially with these cells. The liver is an organ (one of several) in which the parenchymal cells (hepatocytes) are directly accessible for the blood stream. We know, moreover, that the liver cells are the location of many diseases [5]. Development of methods of effective delivery of drugs to the liver cells is thus an important problem.

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