STATE OF THE ANTIOXIDATION SYSTEM DURING METRAZOL KINDLING IN RATS

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Disturbances of regulation of lipid peroxidation (LPO) are among the main components of the pathogenesis of certain forms of epilepsy [1-3, 7]. As one possible cause of the disturbances of LPO regulation in epileptogenesis the authors previously suggested the development of insufficiency of the antioxidative system (AOS) [9]. However, the acute development of bemegride-induced convulsions in rats was not accompanied by any decrease in activity of the components of AOS in the brain [9]. At the same time it has been shown that preliminary injection of superoxide dismutase (SOD) considerably weakened epileptic activity (EA) induced in rats by metrazol [8] or penicillin [4].

To shed further light on the role of AOS in the pathogenesis of EA, we studied the state of AOS in rats during formation of the "kindling" phenomenon, produced by daily injection of metrazol in subthreshold doses (metrazol kindling) and manifested as a progressive increase in sensitivity of the brain to this epileptogen.

EXPERIMENTAL METHOD

Male Wister rats weighing 200-300 g were kept under ordinary animal house conditions on a standard diet. Kindling was induced by daily intraperitoneal injection of metrazol in a subthreshold dose (30 mg/kg). The corresponding volume of physiological saline was injected into control animals. The intensity of seizure activity was assessed by a five-point system: 0 points) absence of seizure response, 1 point) twitching of the head or individual trunk muscles, 2 points) clonic convulsions of the forelimbs, 3 points) clonic convulsions of the whole trunk, 4 points) clonicotonic convulsions with the animal falling on its side and postictal depression, 5 points) repeated marked clonictonic convulsions sometimes followed by death of the rat [10].

The rats were decapitated on the 5th, 17th, and 32nd days. On the day of sacrifice the animals were not given metrazol. Blood was collected in a glass flask containing 3.8% sodium citrate solution, made up in physiological saline. The ratio of blood to anticoagulant solution was 5:1 by volume. The blood was centrifuged in the cold (3000 rpm) and the plasma separated. The erythrocytes were washed 3 times with cold physiological saline and hemolyzed (in samples for determination of SOD activity the hemoglobin was precipitated), as described previously [12]. The hemoglobin concentration in the hemolysate was determined by the method of Drabkin et al., SOD activity in the hemolysate was determined by the method in [11] at 30°C. The quantity of enzyme required to give 50% inhibition of reduction of nitro-BT in formazan under the determination conditions was taken as the units of SOD activity. SOD activity also related to the hemoglobin content of the erythrocytes and protein concentration in the samples after hemoglobin precipitation. Gluthathione peroxidase (GP) activity in the hemolysate was estimated as oxidation of NADPH in a coupled glutathione reductase system, using tert-butyl hydroperoxide as the substrate [6] at 30°C. GP activity was expressed in nmoles NADPH/min/mg hemoglobin. Blood plasma was obtained as described previously [3] and EDTA was added to it up to a final concentration of 5 mM. The samples were kept at −20°C for 2-7 days. The α-tocopherol (TP) concentration was determined in the blood plasma [13].

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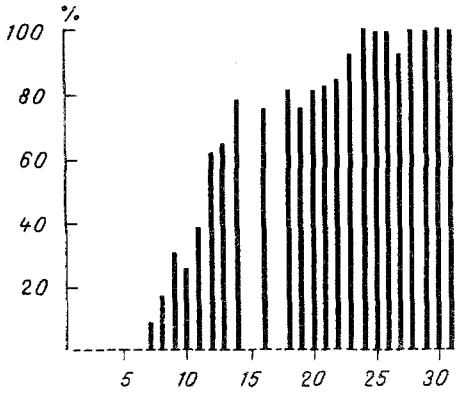

Fig. 1

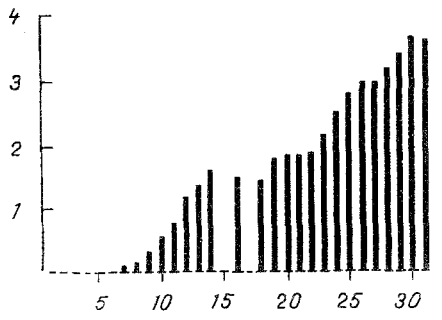

Fig. 2

Fig. 1. Effect of daily injection of subthreshold doses of metrazol on development of predisposition to seizures in rats. Abscissa, time of experiment (in days); ordinate, number of animals with seizure activity (in %).

Fig. 2. Effect of daily injection of metrazol in subthreshold doses on intensity of seizures in rats. Abscissa, time of experiment (in days); ordinate, average intensity of seizures (in points).

Brain hemogenate and its cytosol fraction were prepared for determination of enzyme activity [9]. The TP concentration [13] and SOD and GP activity were determined [11, 6]. Glutathione reductase (GR) activity was measured under the conditions described in [5].

LPO activity in brain homogenate was determined as its content of products reacting with 2-thiobarbituric acid (TBA). For this purpose 1.5 ml of a 30% solution of TCA was added to 0.2 ml of homogenate, the mixture was shaken for 1.5 min, and 1.5 ml of freshly prepared 0.5% TBA was added, after which the sample was incubated at 50°C for 2 h. After cooling, the optical density of the solution was measured at 535 nm, assuming that $E_{535} = 1.56 \cdot 10^{-5}$ $M^{-1} \cdot cm^{-1}$ [14].

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

The protein concentration in the samples was measured by Lowry's method. The following reagents were used: EDTA, xanthine, xanthine oxidase, nitro-BT, TP, stearic acid, glutathione, and TBA were from Serva (West Germany), Tris, GR, and tert-butyl hydroperoxide were from Sigma (USA), NADPH was from Reanal (Hungary), and the other reagents were of the chemically pure grade.

EXPERIMENTAL RESULTS

Daily injection of metrazol in subthreshold doses caused a gradual increase in predisposition to seizures and in the intensity of seizures in the rats (Figs. 1 and 2). Some rats developed the first seizure manifested after the 7th injection of metrazol: initially fewer than 10%, but after 2 weeks, 80% of the rats. The number of animals with seizures and the intensity of the seizures (about 2 points) subsequently remained virtually unchanged until the 21st-22nd day, after which both parameters rose sharply, and by the 25th day of injection metrazol, in a subthreshold dose, caused intensive (on average 3 points) seizures in all rats. At this time clonic convulsions of the whole trunk gave way to marked clonicotonic convulsions, and on the 30th day half of the rats exhibited clonicotonic convulsions with falling on the side (4 points; Fig. 2). This time course of development of metrazol kindling in the rats is in agreement with data published previously [10].

The state of AOS was investigated in three groups of animals, differing in the duration of administration of the convulsant. In group 1, no clinical manifestations of seizure activity were present after four injections of metrazol. In group 2, repeated clonic convulsions of the whole trunk were observed on the 16th day of the experiment. In group 3, clonicotonic convulsions were observed on the 31st day of the experiment, the animal fell on its side, and postictal depression appeared. The value of the parameters of AOS studied (SOD, GP, and GR activity, and TP concentration) are given in Table 1.

As Table 1 shows, no changes in enzyme activity or TP concentration were found in the animals of group 1. The concentration of TBA-reacting products in the brain tissue of the

TABLE 1. Effect of Development of Kindling Phenomenon on Activity of Antioxidative Enzymes and Concentration of TP and LPO Products in Rat Brain and Blood (M ± m)

Experimental conditions	Time of experiment, days	SOD			GP		GR	TP		TBA-reacting products
		brain	blood		brain	blood	brain	blood	brain	blood
Control (7)	5	164,6±5,1	9,0±1,0	392,1±38,9	22,1±0,8	362,9±23,5	16,9±1,1	12,1±0,8	7,3±0,7	0,14±0,01
Metrazol (7)		171,5±4,4	9,2±0,7	403,3±37,3	21,8±1,5	339,3±16,2	19,0±1,2	12,5±0,6	5,7±0,1	0,18±0,01*
Control (7)	17	179,5±2,4	12,0±0,3	471,2±9,9	27,6±1,9	479,3±33,7	19,2±0,9	10,3±0,1	—	0,31±0,01
Metrazol (7)		178,6±6,1	11,7±0,4	482,6±16,2	24,7±1,0	533,7±30,8	20,0±0,7	11,6±3,6	—	0,29±0,02
Control (7)	32	140,9±6,6	3,7±0,3	335,6±19,1	18,1±1,6	718,2±41,6	16,1±0,8	13,5±2,5	1,6±0,2	0,20±0,03
Metrazol (7)		148,0±5,8	3,9±0,3	359,5±28,8	17,8±0,6	590,5±49,7**	15,7±0,7	10,8±1,1	2,2±0,3	0,17±0,01

Legend. SOD activity in brain) in relative units/mg protein, in blood) relative units/mg hemoglobin (numbers on left) and relative units/mg protein (numbers on right); GP activity in brain) nmoles NADPH/mg protein/min, in blood) nmoles NADPH/mg hemoglobin/min; GR activity) nmoles NADPH/mg protein/min; TP concentration) μg/g tissue (brain) or μg/ml plasma (blood); TBA-reacting products) nmoles/mg tissue. Number of animals given in parentheses. *$p < 0.02$, **$p < 0.01$ compared with control.

experimental rats was 22% higher than in the control. In the animals of group 2 also no changes were found in the parameters studied. A tendency for GP activity to fall ($p < 0.1$) was observed in the erythrocytes of the "kindling" rats of group 3. The other parameters did not differ from those in the control.

During the development of metrazol kindling in rats no significant changes thus could be found in the activity of systems participating in LPO regulation. A predisposition for EA induced by metrazol, increasing during chronic administration of the drug, is evidently formed by other mechanisms, unconnected with lipid metabolism in the nervous system. In particular, disturbances of GABA-ergic control may participate in the development of this phenomenon [10]. Meanwhile the seizures themselves and the development of EA in the brain are accompanied by activation of LPO [1-3]. All these data suggests that LPO activation in the epileptic syndrome is linked with the development of EA and hyperactivity in brain structures and is secondary in character, although the pathogenetic importance of this phenomenon for the subsequent development of EA is not in dispute.

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