STATE OF THE ANTIOXIDANT SYSTEM DURING INDUCTION OF PRIMARY GENERALIZED EPILEPTIC ACTIVITY IN RATS

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Induction of epileptic activity (EA) in the cerebral cortex of animals by various epileptogens is accompanied by activation of lipid peroxidation (LPO) in the CNS [4, 5, 9, 18]. Preliminary injection of antioxidants into the animals prevented the LPO activation effect and considerably inhibited EA [4, 8, 9, 17]. On the basis of these data it was concluded that uncompensated activation of LPO plays a pathogenic role in the development of EA [4, 8, 9, 17]. Under normal conditions a stable level of LPO in the body is maintained by function of a multicomponent antioxidant system (AOS) in the body [12]. Uncompensated activation of LPO processes during EA is evidently due to damage to the LPO regulating system, probably as a result of insufficiency of the AOS.

The aim of this investigation was to study the state of the AOS during acute development of primary generalized EA in rats. For this purpose, activity of enzymes of the AOS was investigated in brain tissue: superoxide dismutase (SOD), glutathione peroxidase (GP), and glutathione reductase (GR); the concentration of the principal antioxidant of the body, α-tocopherol (α-TP), also was determined. The α-TP level in peripheral blood plasma also was determined and the acid resistance of the erythrocytes (ARE) assessed.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 150-200 g. Primary generalized EA 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 2-4 min (the period of initial seizure manifestations), 6-8 min (immediately after an attack of generalized convulsions), and 20 min after injection of bemegride. 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 v/v. Blood plasma and a suspension of erythrocytes in isotonic phosphate buffer were obtained as described in [5, 13].

Immediately after decapitation the brain was removed from the rats' skull, separated from the cerebellum, washed in cold (0-4°C) physiological saline to remove blood, and homogenized in a glass homogenizer with Teflon pestle. The homogenization medium, containing 146 mM NaCl, 0.1 mM EDTA, and 20 mM Tris-HCl, pH 7.4 (20°C), was added to the brain in the ratio of 5:1 v/v. To investigate enzyme activity the supernatant obtained after centrifugation of the homogenate for 60 min at 105,000g on a VAC-601 centrifuge (East Germany) was used. The test material was frozen and kept until required for determination of enzyme activity at −14°C. The samples were thawed at 37°C before measurements were made.

SOD activity was determined by the method in [11] under conditions described previously [1]. The unit of SOD activity was taken to be the quantity of enzyme required to inhibit by 50% reduction of nitro-BT into formazan under the conditions of determination. GP activity was estimated as oxidation of NADPH in a coupled glutathione reductase system [14], using tert-butyl hydroperoxide as the substrate [6]. GR activity was studied under conditions de-

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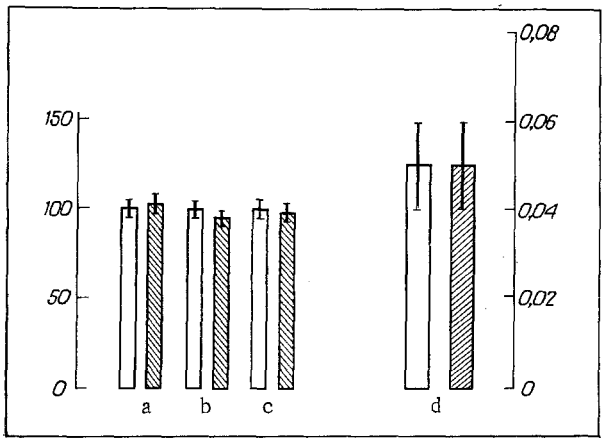

Fig. 1. State of AOS in rat brain 20 min after injection of bemegride into animals. Ordinate: on left) enzyme activity (in %); on right) α-TP concentration (in μg/mg protein). a) SOD, b) GP, c) GR, d) α-TP. Unshaded columns) control; shaded) bemegride.

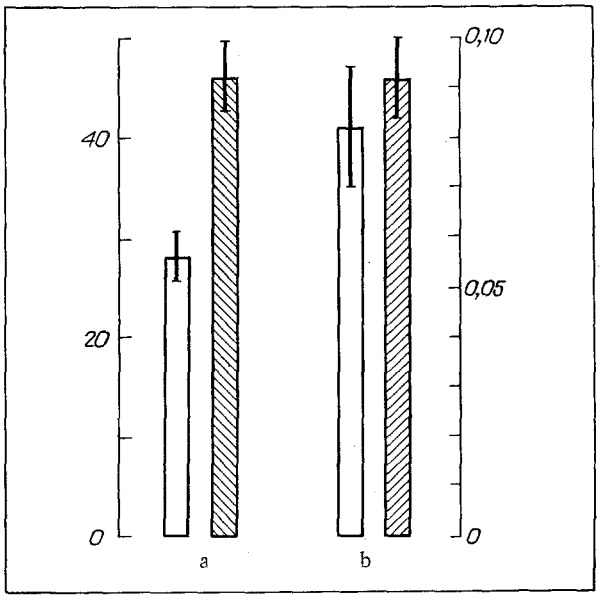

Fig. 2. ARE (a) and α-TP concentration (b) in rats' blood 20 min after injection of bemegride into animals. Ordinate: on left) hemolysis of erythrocytes (in %); on right) α-TP concentration (in μg/mg protein). Remainder of legend as to Fig. 1.

scribed in [6]. GP and GR activity was expressed in nanomoles NADPH/min/mg protein (activity of the AOS enzyme will henceforward be given in activity units). The α-TP concentration was determined by the method in [16]. Optical density was measured on the Hitachi-220 spectrophotometer (Japan) at 37°C. The intensity of fluorescence was measured on a Hitachi MPE-4 (Japan) fluorometer at 25°C.

ARE was determined by the method in [13], by estimating resistance of the erythrocytes to hemolysis induced by the action of definite concentrations of hydrogen peroxide. The protein concentration was determined by Lowry's method.

EXPERIMENTAL RESULTS

Between 5 and 7 min after injection of bemegride into the rats in a dose of 20 mg/kg the animals developed clonicotonic convulsions, with the animal falling on to its side and with a distinct tonic extension phase. It was shown previously [5] that with doses of bemegride of about 20 mg/kg, the level of LPO products in the cerebral cortex and peripheral blood plasma of rats 15-20 min after its injection is 1.5-3 times higher than the control level [5]. Activation of LPO was perhaps associated with insufficiency of the AOS.

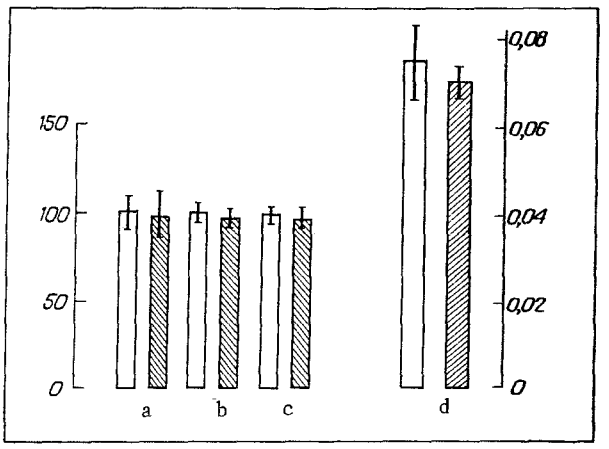

Fig. 3. State of the AOS in rat brain 1-2 min after end of attack of clonicotonic convulsions. Legend as to Fig. 1.

However, the results indicated that 20 min after injection of bemegride, activity of the enzymes of the AOS in the brain and the α-TP concentration in it were unchanged (Fig. 1). The α-TP concentration in the blood plasma also was unchanged under these conditions (Fig. 2). Assuming that changes in the state of the AOS could be compensated during the 10-15 min which elapsed from the time of the generalized seizure until sacrifice of the animal, the parameters listed above were determined immediately (1-2 min) after the end of the clonicotonic convulsions. It was shown that in this case activity of the "antioxidant" enzymes and α-TP concentration in the brain were unchanged (Fig. 3).

Finally, measurement of SOD and GR activity in the period of the initial seizure manifestations (the development of active searching movements of the animals, spasms of individual muscles of the head, neck, and shoulder girdle) before the development of generalized convulsions showed stability of the AOS in this case also. For instance, SOD activity in brain homogenate of the control and experimental animals was 249.0 ± 5.6 and 249.0 ± 10.4 activity units. GR activity in these same groups of animals was 25.6 ± 1.1 and 30.2 ± 4.8 activity units respectively.

Meanwhile ARE 20 min after injection of the convulsant was considerably reduced (Fig. 2). The reduction in ARE probably took place on account of an increase in the concentration of LPO products in the blood plasma [5].

Elevation of the level of LPO products observed in the rat CNS during the development of acute bemegride-induced primary generalized EA, was not connected in all probability with a decrease in activity of the AOS, but was due to activation of reactions involved in the initiation of free-radical conversions of brain lipids. It is a quite difficult task at the present time to determine the source (or sources) of the increase in lipid peroxidation, although some possible approaches to the solution of this problem may perhaps deserve discussion.

In particular, we know that the development of seizures is accompanied by worsening of the intracerebral hemodynamics, which leads to the development of hypoxemia and hypoxia [7]. It has also been shown that cerebral ischemia causes activation of LPO in the brain [19]. The mechanism of this phenomenon has not yet been explained. It is assumed that activation of LPO during tissue hypoxia and ischemia is the result of accumulation of an excess of reduced carriers in the respiratory chain of the mitochondria, which leads to incomplete reduction of the molecular oxygen dissolved in the lipid matrix of the membrane [3]. As a result the concentration of active forms of oxygen, capable of interacting with polyunsaturated fatty acid residues of membrane phospholipids, and thus giving rise to reactions of their free-radical peroxidation, in the cell increases.

At the same time, the possibility cannot be ruled out that activation of LPO during cerebral ischemia or during the development of EA in general is due to the liberation of prooxidants, such as iron, stored in the cell. The results of histopathological investigations also provide evidence of considerable deposition of iron in the form of hemosiderin in foci of hyperactivity in the brain of patients with post-traumatic epilepsy [15], and also re-

sults indicating a significant increase in the iron concentration in the biological fluids of patients with epilepsy [10].

The structural factor likewise must not be forgotten. For instance, it was shown previously that the development of bemegride-induced EA in rats is accompanied by activation of phospholipase hydrolysis in neuronal membranes in the cortex. Activation of phospholipase hydrolysis in membranes, however, is known to disturb the packing of the lipid matrix, which may perhaps facilitate access of LPO initiators to the oxidation substrate, located initially in the depth of the membrane bilayer. Finally, combined action of the above factors with others activating the LPO process is a possibility.

To conclude, it can be stated that the absence of changes in the state of the AOS in rats with bemegride-induced EA may be a distinguishing feature of this particular model of acutely developing EA. To elucidate the role of the AOS in the pathogenesis of EA fully, investigations are needed on other models of EA, including on models of chronic EA.

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