sumption: a relative excess of REM sleep in inactive animals, reflecting their emotional status, is evidently one factor determining the high level of alcohol motivation.

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ANTICONVULSANT ACTION OF SUPEROXIDE DISMUTASE

G. N. Kryzhanovskii, E. V. Nikushkin, I. R. Tupeev, and V. E. Braslavskii

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Research in our own [1, 2, 4] and other [6] laboratories has shown that disturbances in the regulation of lipid peroxidation (LPO) are an important stage in the pathogenesis of certain forms of epilepsy. As one possible cause of disturbances in LPO regulation in epilepsy the writers have suggested the development of insufficiency of the antioxidant system and, in particular, insufficiency of the "antioxidant protection" enzymes [10]. It has been shown that the development of generalized epileptic activity (EPA) in rats is accompanied by a fall in the blood level of superoxide dismutase (SOD) activity [8]. A significant fall in SOD activity and also in the activity of another enzyme of "antioxidant protection" — namely glutathione peroxidase — has been found by the writers also in the blood of patients with generalized forms of epilepsy [8]. Simonyan et al. [9] showed that preliminary administration of SOD to rats considerably weakens the development of metrazol convulsions. These facts confirm the validity of the hypothesis mentioned previously.

When continuing our research in this direction, we studied the effect of SOD on penicillin-induced focal EPA in rats and also on activity of LPO in the brain.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-230 g, kept on a standard diet under ordinary animal house conditions. The development of EPA in the rats' cerebral cortex was induced, just as previously [3], by application of the sodium salt of penicillin to the sensomotor cortex. Under hexobarbital anesthesia two burr-holes 2-4 mm in diameter were drilled in the animal's skull above the sensomotor cortex of both hemispheres and the dura mater was removed from these areas. To record the electrocorticogram (ECoG) silver electrodes were applied to the dura at a distance of 0.2-0.3 mm in front of the burr-hole. The reference electrode was inserted into deep brain structures at the junction between frontal, temporal, and parietal bones of the right hemisphere. The exposed brain surface was moistened with physiological saline and covered with adhesive tape. The experiments began the day after the operation, on unanesthetized animals. A preparation of SOD obtained from bovine erythrocytes (Sigma, USA) was injected intraperitoneally, in physiological saline (1 mg/ml), in a dose of 1 mg/kg body weight 30 min before application of penicillin. Control animals received an injection of the corresponding volume of physiological saline. The ECoG

Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Changes in Focal EPA in Rats after Preliminary Injection of SOD

Experimental conditions	Number of experiments	T_1, sec	T_2, sec	Number of seizures in 1.5 h
Application of penicillin (control)	13	126±12	298±42	36±6
Injection of SOD + application of penicillin	12	265±43*	950±140*	14±2*

Legend. *$p < 0.01$ compared with control.

TABLE 2. Effect of Penicillin on SOD Activity

Experimental conditions	Injection of SOD + application of penicillin			
	0 (10)	10 000 (3)	20 000 (3)	40 000 (3)
Application of penicillin (control)	250.2±3.3	249.1±6.4	254.9±5.7	261.6±14.0

Legend. Number of experiments shown in parentheses.

was recorded on a Nihon Kohden polygraph (Japan). Separate discharges began to appear 2-3 min after penicillin application against the background of the normal ECoG: these were interictal discharges whose amplitude rose rapidly to reach 800-1200 μV. These interictal discharges gradually increased their following frequency on the ECoG and changed into an epileptic seizure — a "burst" of electrical discharges with a frequency of more than 3 discharges per second and with a total duration of about 24 sec [7]. Each epileptic seizure was followed by a refractory period, during which the ECoG was indistinguishable from normal, after which the interictal discharges reappeared and changed into the next epileptic seizure. The number of seizures developing in the first 90 min after application of the convulsant was counted and the time until the appearance of the first interictal discharge (T_1) and to the appearance of the first epileptiform seizure (T_2) was measured [7].

Samples of brain tissue from the focus of EPA (2-3 mg) for determination of the levels of LPO products were taken 90 min after penicillin application. The material was homogenized in a glass homogenizer with 1 mM EDTA at 0-4°C. LPO activity in the cortical homogenate was determined by measuring concentrations of products reacting with 2-thiobarbituric acid (TBA). This 0.5 ml of homogenate was treated with 1.5 ml of a 30% solution of TCA, shaken for 1.5 min, treated with 1.5 ml of freshly prepared 0.5% TBA, and then incubated for 2 h at 50°C. After the solution had cooled, optical density was measured at 535 nm on a Hitachi-320 spectrophotometer (Japan), assuming that $\varepsilon_{535} = 1.56 \cdot 10^5\ M^{-1} \cdot cm^{-1}$ [13].

In experiments to study the effect of administration of exogenous SOD on activity of the endogenous enzyme in the rat cerebral cortex, the SOD preparation was injected intraperitoneally into intact animals in a dose of 1 mg/kg in physiological saline (1 mg/ml). Control animals received an injection of the corresponding volume of physiological saline. The rats were decapitated 30 min after injection of SOD, the brain was removed and washed in cold physiological saline (0-4°C) to remove blood, and the cortex separated from the underlying structures. The cytosol fraction was prepared from the cerebral cortex for SOD assay as described previously [10]. SOD activity was determined by the method in [11]. The unit of SOD activity was taken to be the quantity of the enzyme required to inhibit reduction of nitro-BT into formazan by 50%.

The cytosol fraction of rat cerebral cortex also was used to study the effect of penicillin on endogenous SOD activity in experiments in vitro. Penicillin (final concentration 10,000-40,000 U) was added to the cytosol fraction, which was incubated for 30 min at 37°C, when SOD activity was determined. The protein concentration in the samples was determined by the method of Lowry et al.

The following reagents were used: EDTA, TBA, xanthine, xanthine oxidase, and nitro-BT were obtained from Serva (West Germany); SOD and Tris from Sigma (USA); other reagents were of the chemically pure grade.

EXPERIMENTAL RESULTS

Preliminary (30 min beforehand) injection of SOD into the rats led to an increase in the latent periods of appearance of the first interictal discharge (T_1) and the first seizure (T_2;

Table 1); the number of seizures recorded during 1.5 h of observation was considerably reduced under these circumstances.

Injection of SOD also reduced ($p < 0.05$) the TBA concentration in the cerebral cortex of the animals with seizures.

Because of the insufficient quantity of material sampled it was impossible to estimate changes in endogenous SOD activity in the focus of EPA. Meanwhile injection of SOD into intact animals (in this case SOD activity was determined in the entire cerebral cortex) caused no change in activity of this enzyme in the rat cerebral cortex.

To study the direct effect of the convulsant (penicillin) on SOD, penicillin was added in various concentrations to a solution of the "pure" enzyme in the incubation medium and incubated at 37°C for 30 min. The experiments showed that in the concentrations studied penicillin does not affect SOD activity (Table 2). The first investigation thus showed that the SOD preqaration had a marked anticonvulsant action. It can be postulated that this effect is connected with the effect of the enzyme on LPO: with a decrease in its intensity or, more exactly, with a smaller increase in the intensity of LPO, arising as was shown previously [3, 7] during seizure development. Evidence of inhibition of LPO following injection of SOD was given by the decrease in the TBA concentration in the cortex. To judge from the results of the experiments on intact rats, under normal conditions SOD does not pass through the blood-brain barrier. However, during the development of EPA, permeability of the blood-brain barrier for SOD may increase, as was the case with other macromolecular compounds [12]. It can be tentatively suggested that inhibition of EPA and the decrease in the concentration of LPO products in the cerebral cortex of rats receiving SOD were connected with this fact.

The absence of any effect of penicillin on SOD in the experiments in vitro is evidence that the development of the penicillin-induced focus of EPA was not connected with inhibition of this enzyme.

The acute development of generalized EPA was not accompanied by a decrease in activity of the antioxidant system in the rat cerebral cortex [10]. This means that the cause of LPO activation during EPA is not a decrease in the activity of this system below its initial level, as has been observed in some other forms of CNS pathology [5]. Meanwhile the fact that preliminary injection of SOD reduces EPA suggests that activity of the intrinsic enzymes of the "antioxidant" series may not be sufficient to prevent intensification of LPO and of free-radical damage to neuron membranes during the action of epileptogens.

The results of this investigation are evidence that it is worth while supplementing traditional anticonvulsant therapy by administration of preparations increasing the activity of the antioxidant system of the body.

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