LIPID PEROXIDATION IN A FOCUS OF HYPERACTIVITY IN THE RAT CEREBRAL CORTEX

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It is shown that lipid peroxidation (LPO) can participate in the mechanism of development of paroxysmal activity in the rat cerebral cortex. The appearance of epileptic activity as a result of application of the sodium salt of penicillin to the surface of the sensomotor cortex led to a sharp rise in LPO products in the fraction of unpurified synaptosomes isolated from a focus of hyperactivity. Preliminary injection of the antioxidant α-tocopherol into rats abolished the LPO activation effect and considerably reduced the number of paroxysms recorded on the electrocorticogram during existence of the focus.

KEY WORDS: generator of pathologically enhanced excitation; cortical epileptic focus; penicillin; cortical synaptosomes; lipid peroxidation; α-tocopherol; antiepileptic action.

Much evidence has now been obtained to show that the appearance of epileptic activity due to the formation of a generator of pathologically enhanced excitation [5] in the CNS is connected with sharp changes in the function of neuron membranes, including a marked increase in their permeability for cations and, in particular, for K^+ [11], persisting depolarization, and inactivation of Na,K-ATPase [3, 6]. It has also been shown that one mechanism of the increase in permeability of various model and biological membranes is the intensification of lipid peroxidation (LPO) in them [2, 8]. This fact, together with data on LPO activation in isolated nerve endings (synaptosomes), when depolarized by different methods [7] and on depression of Na,K-ATPase activity in synaptic membranes during intensification of LPO in them [11], suggest a pathogenetic connection between LPO reactions and activity of the generator of pathologically enhanced excitation and, in particular, with epileptic activity.

To test this hypothesis, the state of LPO in the fraction of unpurified synaptosomes (FUS), isolated from the rat cerebral cortex in the region of a focus of hyperactivity, in the absence of and after preliminary injection of an antioxidant, and the effects of the antioxidant on epileptic activity were investigated.

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. Under hexobarbital anesthesia two holes measuring 2-4 mm in diameter were drilled in the animal's skull above the region of the sensomotor cortex of both hemispheres and the dura was removed from these areas. To record the electrocorticogram (ECoG) silver electrodes were placed on the dura 0.2-0.3 mm anteriorly to the hole in the skull. The reference electrode was inserted into deep brain structures at the junction between the front, temporal, and parietal bones of the right hemisphere. The exposed brain surface was moistened with physiological saline and covered with adhesive tape. Experiments were carried out on the day after the operation on unanesthetized animals. The rats were fixed in a special frame allowing movement of the limbs. Pieces of filter paper measuring 1.5×3.5 mm, soaked in penicillin solution (20,000 i.u./ml), were applied to the surface of the sensomotor cortex of both hemispheres [6]. Pieces of filter paper soaked in 0.85% NaCl solution were applied to the control animals.

Brain tissue from the focus of hyperactivity for obtaining FUS was sampled by means of a special cooled spoon with sharpened edges 15 min after application of penicillin, or 10-20 min after disappearance of epileptic activity recorded on the ECoG in the focus. To isolate FUS the method suggested by Hajôs [9] was used, with centrifugation of the postnuclear supernatant for 20 min at 9000g. The resulting pellet was suspended

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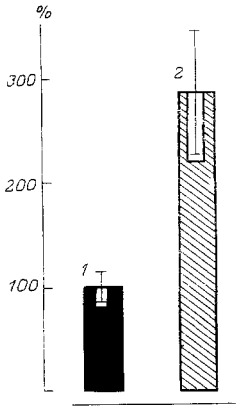

Fig. 1

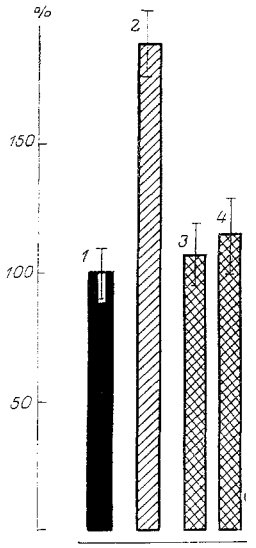

Fig. 2

Fig. 1. Concentration of LPO products in FUS isolated from brain tissue in region of focus of hyperactivity. 1) Control; 2) penicillin. Nine groups of animals investigated in each series. Here and in Fig. 2, concentration of TBA-active products (in %) plotted along ordinate.

Fig. 2. Concentration of LPO products in FUS isolated from brain tissue in region of focus of hyperactivity after injection of α-tocopherol. 1) Control; 2) penicillin; 3) penicillin + 10 mg/kg α-tocopherol; 4) penicillin + 100 mg/kg α-tocopherol. Three groups of animals investigated in each series.

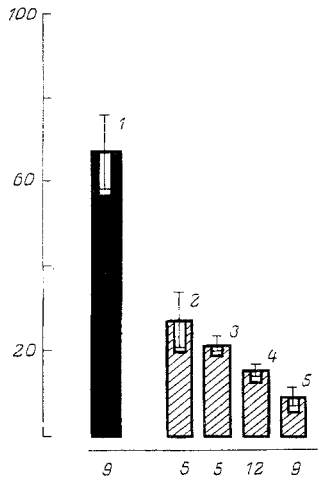

Fig. 3. Effect of α-tocopherol on number of paroxysms recorded on ECoG in focus of hyperactivity during its existence. 1) Penicillin, 2-5) penicillin and α-tocopherol in dose of 0.1, 1, 10, and 100 mg/kg respectively. Abscissa, number of animals investigated in each series; ordinate, number of paroxysms.

in Krebs–Ringer medium containing (in mM): NaCl 132, KCl 5, NaH_2PO_4 1.2, glucose 10, $CaCl_2$ 1.2, $MgCl_2$ 1.3, Tris-HCl, pH 7.6 (20°C) 20. LPO activity in FUS was determined by measuring the concentration of products reacting with 2-thiobarbituric acid (TBA-active products) [2]. Control and experimental groups (3-4 rats in each group) of approximately the same weight and age, receiving the same diet, and used in the experiments at the same time, were compared.

The antioxidant used was α-tocopherol in a dose of between 0.1 and 100 mg/kg body weight. A solution of α-tocopheryl acetate (from Sigma, USA) in ethanol was injected intraperitoneally in a dose of 0.1 ml 24 h before the experiment. After injection, the α-tocopheryl acetate is hydrolyzed in vivo (in the course of 24 h) to free tocopherol [1].

EXPERIMENTAL RESULTS

Results of determination of TBA-active products in FUS isolated from rat cerebral cortex taken from the region of a focus of hyperactivity, or the corresponding part of the cortex of control animals, are given in Figs. 1 and 2. The brain tissue was sampled 15 min after application of penicillin, in the stage of marked paroxysmal activity (Fig. 1) or 10-20 min after its disappearance (Fig. 2). The duration of existence of the focus of hyperactivity was counted from the appearance of the first interictal discharge on the ECoG after penicillin application until disappearance of the last. It was usually 2-2.5 h. The development of a focus of hyperactivity in the cortex led to a sharp increase, by 2-3 times compared with the control, in the concentration of TBA-active products in FUS ($P < 0.01$), suggesting activation of LPO in FUS. The quantity of TBA-active products found in the right and left hemispheres was virtually the same in both the control and experimental animals. The addition of penicillin in a concentration of 1×10^{-6}–1×10^{-3}M to the suspension of FUS in Krebs–Ringer medium and incubation of the mixture for 15 min at 37°C (6 experiments) did not affect the content of LPO products in that system. Consequently, the data given above on activation of LPO in a focus of hyperactivity cannot be explained by the direct action of penicillin on the intensity of LPO reactions in FUS of the cerebral cortex. The intensification of LPO observed was thus directly connected with the development of epileptic activity in the animals. Experiments by other workers [4] have shown an increase in the concentration of lipid peroxides and a fall in the level of antioxidative activity in brain homogenates of rats with audiogenic myoclonus; activation of LPO in this case was regarded as the possible cause of the epileptic discharges.

However, the changes in the intensity of LPO reactions in the cerebral cortex during the development of a focus of epileptic activity could be a coupled process – a consequence of the principal stages of formation of a generator of pathologically enhanced excitation. In this connection, the effect of the antioxidant α-tocopherol on the biochemical and electrophysiological properties of the focus of hyperactivity was studied.

Experiments showed that preliminary injection of α-tocopherol in doses of 10 or 100 mg/kg prevented the increase in the concentration of TBA-active products in FUS from cerebral cortex in the region of a focus of hyperactivity (Fig. 2). It was shown that injection of α-tocopherol reduces the number of focal epileptiform paroxysms recorded in rats on the ECoG during the existence of a focus of hyperactivity (Fig. 3) and accompanied by motor activity of the animal.

The appearance of a generator of pathologically enhanced excitation in the rat cerebral cortex in the form of a focus of epileptic activity was thus accompanied by activation of LPO in FUS isolated from the cortex in the region of that focus. Preliminary injection of the antioxidant (α-tocopherol) into the animals abolished the effect of LPO activation and considerably weakened epileptic activity. It can be concluded from the results that LPO reactions participate in the mechanisms of development of epileptic activity and offer new prospects for the development of anticonvulsive therapy.

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EFFECT OF BIORESOLVING MICROSPHERIC PREPARATIONS OF IMMOBILIZED FIBRINOLYSIN ON THE FIBRINOLYSIS SYSTEM

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The effect of fibrinolysin, immobilized on microspherical carriers, on the fibrinolytic system of the blood was studied in dogs. A marked increase in fibrinolytic activity of whole blood was found at the site of deposition of the preparation on account of the enzyme introduced and increased activity of plasminogen activator. Administration of immobilized fibrinolysin leads to a more marked increase in activator activity than of plasmin activity. The results suggest the therapeutic value of this method of administration of the thrombolytic preparation.

KEY WORDS: blood fibrinolysis; plasminogen activator; thrombolytic preparations.

The creation of bioresolving microspheric preparations containing immobilized drugs and, in particular, fibrinolysin, provides a completely fresh approach to the treatment of thrombosis, thromboembolism and possibly, of ischemic heart disease [2-4].

The suggested method of administration of thrombolytic preparations enables the preparation to be deposited (as microgranules with a particle size of 20-40 μ) in the affected blood vessel, a high local concentration of the lytic agent to be maintained near the thrombus, and the degree of contact with natural inactivators of the preparation and the doses used to be reduced, which in turn reduces the immune response of the recipient.

Advances in the method of selective angiography not only enable the location of the thrombosis to be determined accurately, but at the same time, the immobilized preparation can be applied in its vicinity.

The aim of the present investigation was to assess the specific action of fibrinolysin, immobilized on microspheric carriers, on the blood fibrinolysis system under experimental conditions.

EXPERIMENTAL METHOD

Fibrinolysin immobilized on modified Sephadex, with a carrier granule size of 20-40 μ and with a total biological resolving time under physiological conditions of not more than 3 h, was obtained by the method described by Torchilin et al. [3]. In this way preparations of the immobilized enzyme containing active protein in a concentration of 10-80 mg/g carrier were obtained.

Experiments were carried out on nine mongrel dogs weighing 15-20 kg; the femoral artery and vein were isolated under morphine-pentobarbtial anesthesia and the distal and proximal segments of the vein and the abdominal aorta were catheterized. Ten mg of the preparation, containing not less than 1500 units fibrinolysin dissolved in 3 ml rheopolyglucin, was injected into the distal segment of the femoral artery. Blood samples

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