THE ANTICONVULSANT EFFECT OF THE ANTIOXIDANT IONOL

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Evidence has recently been obtained of intensification of lipid peroxidation (LPO) in cell membranes in various pathological processes [1, 8, 9]. The writers found marked activation of LPO previously in foci of epileptic activity in the cerebral cortex [6]. Preliminary administration of the antioxidant α-tocopherol to animals prevented the effect of LPO activation in the focus and depressed epileptic activity considerably [6]. These results led to the conclusion that intensification of LPO plays a pathogenetic role in the mechanisms of epileptogenesis and, consequently also in the mechanisms of formation of generators of pathologically enhanced excitation, of which the epileptic focus is a special case [5].

The object of this investigation was to study the possibility of using other antioxidants as anticonvulsants. The effect of ionol on epileptic activity in rats was studied for this purpose.

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. A focus of epileptic activity was produced by application of the sodium salt of penicillin to the surface of the sensomotor cortex of the animals' brain. Methods of biochemical and electrophysiological measurement were as described previously [6]. Brain tissue from the primary and symmetrically opposite foci of hyperactivity was taken 10-20 min after disappearance (as shown by electrocorticography) of epileptic activity (interictal discharges) in the focus. LPO activity in the fraction of unpurified synaptosomes (FUS) was determined by measuring the concentration of products reacting with 2-thiobarbituric acid (TBA-active products) [2]. Synaptosomes were first suspended at 4°C in Krebs–Ringer solution of the following composition (in mM): NaCl 132, KCl 5, NaH_2PO_4 1.2, $CaCl_2$ 1.2, $MgCl_2$ 1.3, glucose 10, Tris-HCl 20; pH 7.6 (20°C).

Ionol (butylhydroxytoluene, 4-methyl-2,6-di-tert-butylphenol) was injected as a 96% solution in ethanol (0.1 ml) intraperitoneally or subcutaneously once daily on three consecutive days, the last injection being given 24 h before application of penicillin, or as a single injection 24 h before the experiment. The following doses of ionol were used: 1, 10, and 100 mg/kg.

EXPERIMENTAL RESULTS

The results of measurement of the concentrations of TBA-active products in FUS isolated from the cerebral cortex of the rats, in the regions of the primary focus of epileptic activity and the symmetrically opposite, "mirror" focus, are given in Fig. 1. Two groups of animals were tested: control, i.e., not receiving preliminary antioxidants, and experimental, i.e., after injection of ionol.

It was mentioned above that the development of a focus of epileptic activity in the cerebral cortex of rats leads to a two-threefold increase in FUS isolated from the cortex in the region of the focus, and in the concentration of TBA-active products, which is normally 500 ± 100 nmoles/mg protein [6]. Under these circumstances the increase in concentration of TBA-active products was practically identical in the primary and "mirror" foci (Fig. 1) and was independent of the hemisphere (right or left) to which the penicillin was applied. This fact is evidence that penicillin has no direct pro-oxidant action in vivo. The writers showed previously that penicillin, in concentrations of $1 \cdot 10^{-6}$–$1 \cdot 10^{-3}$ M, likewise does not affect the intensity of LPO in vitro (suspension of synaptosomes).

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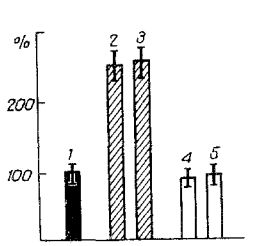

Fig. 1

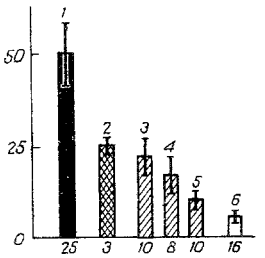

Fig. 2

Fig. 1. Effect of ionol on concentration of LPO products in FUS isolated from cerebral cortex in region of primary focus (2, 4) and "mirror" focus (3, 5) of hyperactivity. 1) Control; 2, 3) penicillin; 4, 5) penicillin + ionol, 100 mg/kg daily for 3 days. Seven animals tested. Ordinate, concentration of TBA-active products (in percent).

Fig. 2. Effect of ionol on number of convulsive paroxysms recorded on ECoG in focus of hyperactivity. 1) Penicillin; 2) penicillin + ionol, 100 mg/kg subcutaneously on three consecutive days; 3, 4, 5) penicillin + ionol, 1, 10, and 100 mg/kg intraperitoneally respectively; 6) penicillin + ionol, 100 mg/kg intraperitoneally daily for 3 days. Abscissa, number of animals tested; ordinate, number of convulsive paroxysms.

Preliminary intraperitoneal injection of ionol in a dose of 100 mg/kg daily on three successive days prevented the activation of LPO observed in membranes of isolated nerve endings extracted from the cortex in the region of the primary and "mirror" foci of hyperactivity (Fig. 1). A similar result was obtained previously with another antioxidant, α-tocopherol [6].

Investigation of the effect of preliminary injection of ionol in parallel experiments on the intensity of epileptic activity in rats, reflected in the ECoG, revealed a marked decrease in the number of focal epileptiform paroxysms recorded in the animals (Fig. 2). The paroxysms were counted during the period of existence of the focus of hyperactivity, usually 2-2.5 h. It should be noted that the discharge patterns of the primary and mirror foci recorded on the ECoG were identical.

The maximal anticonvulsant effect was observed after three intraperitoneal injections of antioxidant in a dose of 100 mg/kg. The number of focal epileptiform paroxysms recorded was reduced in this case by an order of magnitude (Fig. 2). Triple subcutaneous injections (100 mg/kg body weight) or a single intraperitoneal injection of ionol in doses of 1, 10, and 100 mg/kg were considerably less effective (Fig. 2).

Hence the antioxidant ionol, which has already been used for some time in the food industry [7], like α-tocopherol, has a marked anticonvulsant action. This action is evidently due to the ability of ionol to protect cell membranes against the consequences of activation of LPO in them. Intensification of LPO is known to lead to disturbance of the basic functions of the membrane, primarily its barrier (permeability to various substances is increased [4]) and enzymic (activity of membrane-bound enzymes is reduced or even completely inhibited [4, 10]) functions. It is these functions of cell membranes that are mainly upset during the development of foci of epileptic activity in the cerebral cortex [3].

It can be expected that other antioxidants will also prove to have a definite anticonvulsive action. Special interest from this point of view is attached to the study of the pharmacological properties of nontoxic antioxidants, especially those which have already found application in the food industry. Such investigations could lead to the development of new drugs and their application to clinical practice.

Further confirmation has thus been obtained of the hypothesis expressed previously regarding the role of LPO in epileptogenesis [6]. The antioxidant ionol has been shown to possess marked anticonvulsant activity.

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ACTION OF ODIFALINE AND DIFRIL ON THE CATECHOLAMINE CONTENT IN ADRENERGIC NERVE FIBERS IN VARIOUS RAT ORGANS

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Odifaline – 3-phenyl-3-O-hydroxyphenyl-N-(phenylisopropyl)propylamine – is an original coronary dilator, structurally similar to difril,* which has been used in the treatment of angina pectoris and myocardial infarction [11]. The properties of difril, according to data in the literature, are due to its mobilizing action on catecholamine reserves in adrenergic nerves [4, 6] and to its ability to inhibit transmembrane transport of Ca^{++} ions [8]. The action of odifaline and difril has been studied mainly in tissue homogenates by the use of biochemical methods [1, 9], and to a lesser degree, radiographically; only isolated studies have been undertaken by histochemical methods [8, 12, 13]. However, despite their great value, these investigations do not reflect the dynamics of action of the drugs.

It was accordingly decided to study the dynamics of action of difril and odifaline on the catecholamine content in peripheral adrenergic nerves of various organs with different types of sympathetic innervation.

EXPERIMENTAL METHOD

Experiments were carried out on 200 male Wistar albino rats weighing 150-200 g. A suspension of odifaline or difril, made up in a 0.5% solution of carboxymethylcellulose, was injected intraperitoneally in a dose of 50 mg/kg. The animals were killed 1, 6, 12, 18, 24, and 30 h after a single injection of the drug; 6 to 10 animals were investigated at each time. Rats receiving an intraperitoneal injection of a 0.5% solution of car-

*Prenylamine.

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