cytochrome C-reductase and its response to oxygen deficiency in WR and HR rats; this fully corresponds to the data on the role of the NADH-oxidase pathway of energy-rich substrate oxidation in the formation of timely compensatory mechanisms during hypoxia and the regulation of individual brain resistance to it.

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Antioxidant Activity of Anticonvulsive Drugs

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Recent studies have aimed at elucidating the molecular mechanisms of action of preparations used in anticonvulsive therapy [6,15]. This is explained by the inadequacies of current treatments for epilepsy and various convulsive syndromes [13]. It was established in the last decade that disturbed regulation of lipid peroxidation (LPO) is an important component in the pathogenesis of epilepsy [2,5,7-11,14]; one of the proofs of this thesis is the demonstrated marked anticonvulsive effect of antioxidants belonging to various classes of chemical compounds [1,3,4,7,9,10,12]. Therefore, at least for some of the known anticonvulsive agents, a relationship between the molecular mechanisms of anticonvulsive action and the system of LPO regulation may be assumed.

In order to test this hypothesis, we studied the effect of anticonvulsive preparations belonging to dif-

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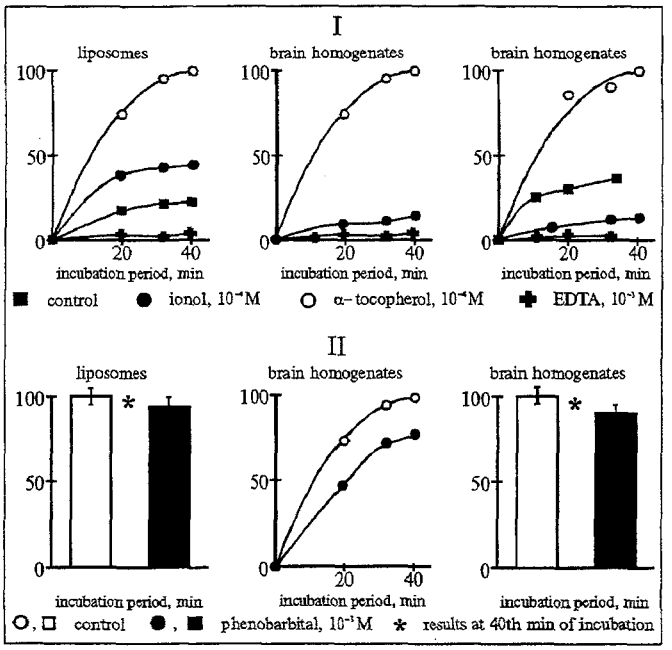

Fig. 1. Effect of ionol, α-tocopherol, and EDTA (*I*), as well as phenobarbital (*II*) on ADLPO and ELPO in the model systems. Here and in Fig. 2, 3 ordinate: TBAP level (% of maximal TBAP level in the control at 40th min of incubation).

ferent chemical classes on the system of LPO regulation in model biological systems.

MATERIALS AND METHODS

Four compounds were chosen from the ones most commonly used in the world health service [6]: phenobarbital (5-ethyl-5-phenylbarbituric acid, Polfa, Poland), diphenin (5-diphenylhydantoin, Serva, Germany), phenazepam (7-bromine-5-(o-chlorphenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-OH, pharmacopeic product, Russia), and Na valproate (sodium propyl-2-pentanoate, Leiras, Finland). The final concentrations of the above compounds in the model systems were 10^{-7}-10^{-3} M. The following control antioxidants were also used: α-tocopherol (10^{-4} M), ionol (10^{-4} M), and EDTA (10^{-3} M). EDTA and Na valproate were dissolved in water before being added to the incubation medium. The other compounds were introduced in the form of ethanol solutions; after their addition the final concentration of ethanol in the incubation medium did not exceed 0.2%.

The following model systems were used: 1) homogenates of rat brain (10-20 mg brain tissue in 1 ml of an aqueous solution containing 146 mM NaCl and 20 mM Tris-HCl, pH 7.4 at 20°C); 2) suspensions of liposomes formed of egg lecithin (2.5 mg lecithin in 1 ml of an aqueous solution containing 100 mM glycine-HCl and 150 mM KCl, pH 2.0 at 20°C). The liposomes were produced by the rapid introduction of 10% egg lecithin dissolved in ethanol into the incubation medium (glycine-buffered solution) at 37°C. The liposome suspension was further preincubated for 2 hours with constant stirring.

In both model systems nonenzymatic ascorbate-dependent LPO (ADLPO) was induced by the addition to the incubation medium of $FeCl_2$+ascorbic acid mixture up to a final concentration of 10^{-5} M and $2 \cdot 10^{-4}$ M, respectively. In addition, in the brain homogenates an enzymatic, NADPзH-dependent LPO (ELPO) was induced by the addition of $FeCl_2$ +NADPзH up to a final concentration of 10^{-5} M and 2×10^{-4} M, respectively.

The experimental study of the antioxidant activity of the tested preparations was organized as follows: the model system (1-3 ml) was preincubated in the corresponding medium at 37°C with continuous stirring for 2-5 min, after which the test preparation was added (a corresponding solvent in equal quantity was added to the control samples). The mixture was stirred for 1 min, after which the ELPO or ADLPO initiators were added. At certain intervals (5-10 min) samples were taken from the incubation mixture and the LPO products in them were determined. The LPO process in the samples was terminated by their being placed in a 1mM EDTA-containing medium. The level of LPO products in the samples (products reacting with thiobarbituric acid, TBA-active products, or TBAP) was recorded as described earlier [11].

The data are presented in the form of characteristic kinetic curves, each of which depicts the results of 6-12 repeated experiments.

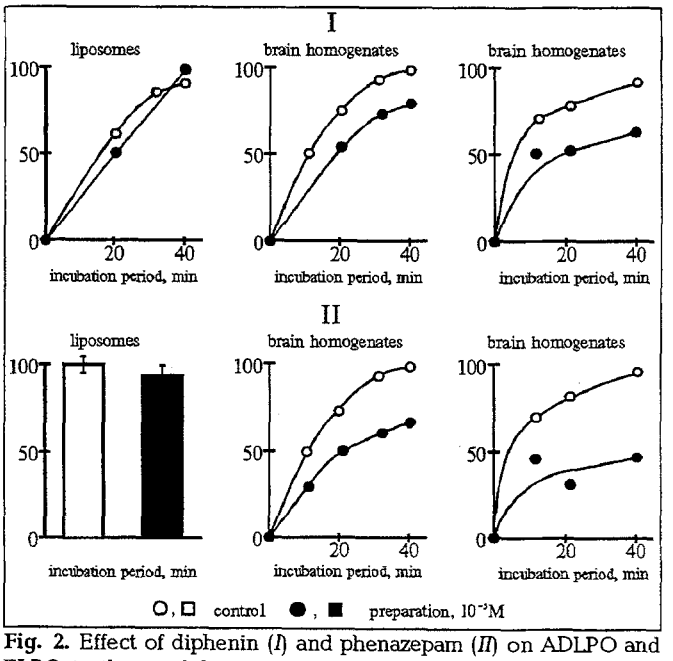

Fig. 2. Effect of diphenin (*I*) and phenazepam (*II*) on ADLPO and ELPO in the model systems.

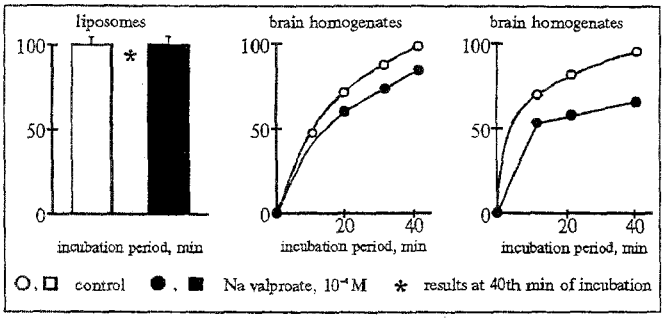

Fig. 3. Effect of Na valproate on ADLPO and ELPO in the model systems.

RESULTS

As mentioned above, we used the well-recognized antioxidants α-tocopherol and ionol, and the complexone EDTA as reference preparations. Both antioxidants and EDTA given in concentrations of 5×10^{-5} M and higher markedly inhibited both ADLPO in the liposomes and brain homogenate, and ELPO in the brain homogenate (Fig. 1). Moreover, EDTA proved to be a more potent LPO inhibitor that α-tocopherol and ionol, producing a 100% inhibition of LPO in all three systems tested when given in a concentration of 10^{-3} M. This fact is of considerable methodological importance, as it permits the use of EDTA for rapid blocking of LPO.

It was shown that phenobarbital in the range of concentrations 10^{-7}-10^{-3} M fails to influence ADLPO in the liposomes and ELPO in brain homogenates (Fig. 1). At the same time, phenobarbital in the maximum concentration tested (10^{-3} M) produced a 20% inhibition of ADLPO in the brain homogenates (see Fig. 1).

Diphenin in concentrations of 10^{-6}-10^{-4} M exerted no effect on ADLPO in liposomes and inhibited LPO processes of both types in the brain homogenate, ADLPO by 20% and ELPO by 40% (Fig. 2).

Phenazepam in concentrations of 10^{-7}-10^{-3} M, like diphenil, produced no effect on ADLPO in the egg lecithin liposomes and inhibited ADLPO and ELPO in the brain homogenate by 30% and 50%, respectively (Fig. 2).

Finally, Na valproate in a concentration up to 10^{-4} M did not alter ADLPO in the liposomes and brain homogenate, and reduced ELPO in the brain homogenate by 25% (Fig. 3).

Thus, apparently not one of the four tested compounds is a true antioxidant, as the compounds had no effect on ADLPO in a simple nonmetabolizing system - liposomes - in contrast to α-tocopherol and ionol. At the same time, phenobarbital and Na valproate inhibited respectively ADLPO and ELPO in the brain homogenate, while diphenin and phenazepam led to a reduction of both LPO processes in this system. An LPO-inhibiting effect of other barbiturates, specifically thiopental, in brain homogenate was found earlier by Smith and coworkers [16]. The effect of these preparations on the rate of TBAP accumulation in brain homogenate is apparently due to their influence on certain components of the LPO-regulating system in the nervous tissue, this effect possibly being indirect. The specific targets for these preparations are undefined as yet. One may only presume that the targets for phenobarbital, diphenin, and phenazepam are different from those for Na valproate.

Thus, the results obtained show an LPO-inhibiting effect in the nervous tissue of the drugs widely used in anticonvulsive therapy. Perhaps this property enhances or even to some extent causes their antiepileptic effect. At any rate, the results of this investigation present substantial additional confirmation of the important role of uncompensated LPO activation in the central nervous system for the pathogenesis of epilepsy.

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