EFFECT OF CENTRAL CHOLINOLYTICS ON LIPID PEROXIDATION

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Antioxidants, which are being used on an ever widening scale in medicine, are found among many classes of compounds, both natural [3] and synthetic [2, 15] in origin.

It was accordingly decided to study the antioxidative properties of central cholinolytics, drugs with high biological activity and belonging to various groups of chemical compounds, for some of them are known to possess membrane-stabilizing properties [11]. Research of this kind is also interesting because it may help to explain effects of cholinolytics unconnected with acetylcholine receptor blockage.

EXPERIMENTAL METHOD

The effect of drugs on lipid peroxidation (LPO) processes *in vitro* was judged by their ability to inhibit by 50% the accumulation of products reacting with thiobarbituric acid (TBA) in a homogenate of rat brain, incubated for 10 min at 37°C. LPO was initiated by adding Fe^{++} and ascorbic acid (final concentration 10^{-6} M) to the homogenate. TBA-active products were determined by the method [4]. Antioxidative activity (AOA) in experiments *in vivo* was estimated as the degree of inhibition by the drugs of LPO in the brain and liver of rats poisoned by intraperitoneal injection of CCl_4 in a dose of 3 ml/kg. The antioxidants were injected twice in a dose of 25 mg/kg each time: the first time 1 h before poisoning, the second time 1 h before sacrifice of the animals. Ionol was injected intraperitoneally and pediphen intramuscularly.* The rats were decapitated 24 h after poisoning, and the intensity of LPO was determined in brain and liver homogenate by assay of diene conjugates [10] and of TBA-active products. The central nicotinic (N) and muscarinic (M) cholinolytic activity of the drugs was studied in experiments on albino mice by their ability to prevent nicotine convulsions or arecoline tremor [5].

The central M-cholinolytics atropine, benactyzine, and glipin [8], the central N-cholinolytics pediphen [12], IEM 506 [1], and methyl-tert-butylamminoethyl ester of benzylic acid hydrochloride (MTB), which has marked central M- and N-cholinolytic activity [6], and also the antioxidants — synthetic) ionol, and natural) α-tocopherol — were used. For the experiments *in vitro* solutions of the cholinolytics were made up in Tris-HCl buffer (pH 7.4), and of ionol in ethanol. For the experiments *in vivo*, solutions of the cholinlytics were made up in physiological saline, and ionol in a 1% solution of Tween-20. Equal amounts of the solvents were added to the incubation mixture or injected into the control animals.

*Ionol is 2,6-di(tert-butyl-4-methylphenol); pediphen is 1,1-diphenyl-5-diethylaminopentane.

TABLE 1. Effect of Pediphen and Ionol on LPO in Brain (in optical density units) of Rats Poisoned with CCl_4 in a Dose of 3 ml/kg (M ± m)

Parameter	Control	CCl_4	Pediphen	Ionol
Diene conjugates	0,902±0,260	1,234±0,226	0,822±0,261	0,850±0,280
TBA-active products	0,197±0,012	0,278±0,060*	0,117±0,008*	0,134±0,009*

Legend. *$p < 0.05$ compared with control.

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TABLE 2. Prevention of Nicotine Convulsions in Albino Mice by Ionol (30 mg/kg) and Pediphen (30 mg/kg) (M ± m)

Time of prevention, h	Index of protection by ionol	ED_{50} of nicotine (+ pediphen)/ED_{50} of nicotine alone
1/2	1,28±0,17	2,98±0,29
1	1,71±0,25	1,84±0,23
2	1,36±0,24	1,64±0,21

EXPERIMENTAL RESULTS

The results obtained *in vitro* indicate that central cholinolytics can inhibit LPO in the rat brain ($p < 0.05$). The acetylene amine pediphen had stronger antioxidative activity. Central M-cholinolytics, even in a concentration of 1000 μM, had no effect on LPO processes, in agreement with data in the literature [9]. In the case of MTB, which has both central M- and N-cholinolytic activity, the antioxidative effect was weak (ED_{50} = 700 ± 15 μM). These results suggest that the membrane-stabilizing activity of the central N-cholinolytics during hypo-osmotic hemolysis of erythrocytes [11] is linked with their antioxidative activity.

In CCl_4 poisoning pediphen and ionol inhibited LPO activity in the rat brain equally (Table 1). Neither pediphen nor ionol, in the doses tested, had any effect on LPO in the liver of the poisoned animals.

Since, as the experiments showed, central N-cholinolytics can reduce the intensity of LPO *in vitro* and *in vivo*, it was interesting to study the effect of the antioxidant ionol on the central M- and N-cholinergic systems. It was found that this substance, like pediphen, has no M-cholinolytic activity, but at the same time it exhibits N-cholinolytic activity ($p < 0.05$), although it is 1.5-2 times weaker in this respect than pediphen (Table 2).

Thus, for the first time, we found that central N-cholinolytics possess antioxidative activity, evidence that LPO processes and changes in the functional state of biomembranes connected with them play an important role in the work of the cholinergic system. The AOA of cholinolytics is evidently associated with the fact that they contain an electron-donating amino group [7]. The absence of AOA of the M-cholinolytics, which also are amines, indicates that different substituents present in the molecules of M- and N-cholinolytics have different effects on the electron-donating properties of these substances.

Prevention of the development of nicotine convulsions by the antioxidant ionol may be due both directly to blockage of N-cholinergic receptors by ionol [13] and to its action as an inhibitor of LPO. In the latter case the antagonism between ionol and nicotine can be explained either by its membrane-stabilizing properties or by its ability to reduce disulfide bonds in the active center of the nicotinic acetylcholine receptor [14].

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