EFFECT OF ISCHEMIA AND REPERFUSION OF THE RAT BRAIN ON LIPID PEROXIDATION AND THE PROTECTIVE EFFECT OF ANTIOXIDANTS

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Lipid peroxidation (LPO) has been shown to play an essential role in the pathogenesis of ischemic and reperfusion injuries to various organs [1, 2, 5]. However, the role of LPO processes in cerebral ischemia has been studied only on models of short-term mechanical asphyxia or clinical death [7, 8], in partial focal cerebral ischemia [6, 15], or on a model of decapitation, excluding any possible further reperfusion of the brain [14].

The aim of this investigation was to study the time course of changes in LPO in the rat brain after total ischemia and reperfusion, and also to evaluate the anti-ischemic effect of two synthetic antioxidants, namely ionol and diludin.

EXPERIMENTAL METHODS

The investigation was conducted on 179 male Wistar rats weighing 150-200 g. Cerebral ischemia was created under hexobarbital anesthesia (100 mg/kg) by compressing both carotid and subclavian arteries under an operating microscope for periods of between 5 and 60 min. The completeness of ischemia arising in the brain by this method is demonstrated by the fall of pressure in the distal segments of the carotid arteries to 0, the appearance of a zero line on the EEG after 20-30 sec, cessation of spontaneous breathing of the rats after 4 min, and absence of appearance of contrast material in the blood vessels of the brain and neck distally to the point of occlusion [9]. Reperfusion of the brain was carried out after 30 min of ischemia for periods of 5, 20, and 60 min. Artificial ventilation of the lungs was maintained by the EPM-2 apparatus in the ischemic and postischemic periods.

Brain samples were taken through a burr-hole in anesthetized animals without ischemia (initial data) and also after assigned periods of ischemia and reperfusion. The brain was frozen in liquid nitrogen and homogenized by grinding the frozen tissue in a porcelain mortar.

Table 1. Concentrations of LPO Products in Rat Brain after Ischemia and Reperfusion

Parameter	Duration of ischemia, min					Duration of reperfusion after 30 min of ischemia, min		
	0	5	15	30	60	5	20	60
DC	0,302±0,046	0,457±0,082	0,386±0,085	0,417±0,008*	0,309±0,038	0,426±0,063	0,545±0,055***	0,441±0,041
KD	0,063±0,018	0,118±0,031	0,115±0,009*	0,134±0,010**	0,154±0,029*	0,119±0,016*	0,157±0,025**	0,112±0,007*
MDA	13,17±1,39	21,49±2,09**	23,77±2,69**	25,78±1,96**	23,10±2,47**	28,50±3,93**	26,32±1,36***	23,22±2,18***

$p < 0,05$

Legend. Concentrations of DC and KD given in optical density units/mg lipds, MDA in nanomoles/milligram wet weight of tissue. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with initial level; $p < 005$ compared with 30 min of ischemia without perfusion. Average data for 4-9 experiments are shown.

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Table 2. Effect of Antioxidants Ionol and Diludin on Survival of Animals with Cerebral Ischemia

Period of ischemia, min	No. of animals suviving after injeciton of compound 4 h before ischemia, %					
	without compound (control)	dose of ionol, mg/kg			dose of diludin, mg/kg	
		60	120	240	120	240
10	100	—	—	—	—	—
15	30	—	—	—	—	—
20		—	—	—	73[a,c]	
25	0	—	82[a,c]	—	33	44[b]
30	—	30	44[b] (38[b])	40[b] (42[b])	7	—
35	—	—	9	—	—	—

Legend. Numbers in parentheses are same parameters when compound was injected 24 h before ischemia. [a]$p < 0.05$ compared with 15 min of ischemia in control, [b]$p < 0.05$ compared with 25 min of ischemia in control, [c]$p < 0.01$ compared with 25 min of ischemia in control.

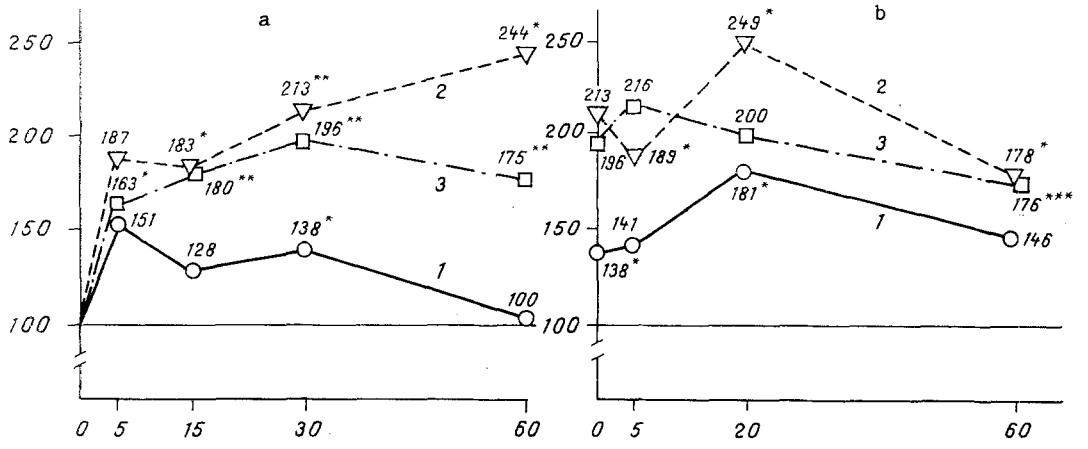

Fig. 1. Concentrations of LPO products (in % of initially) in rat brain after ischemia (a) and reperfusion (b) for various periods. Abscissa, time of ischemia and reperfusion (in min). 1) DC; 2) KD; 3) MDA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with initial level.

Lipids were isolated in the cold from the homogenate by the method in [10]. The extracting mixture was ventilated with specially pure nitrogen and ionol (10^{-5} M) was added. Concentrations of primary (diene conjugates – DC) and secondary (ketodienes – KD) LPO products in the lipids were measured by a spectrophotometric method [11] and the malonic dialdehyde (MDA) content was assessed by the method in [13].

Ionol (2,6-di-tert-butyl-4-methylphenol) was used in doses of 60, 120, and 240 mg/kg, and the dihydropyridine derivative diludin (synthesized at the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR and generously provided by Professor G. Ya. Dubur) was used in doses of 120 and 240 mg/kg. The compounds, in the form of oily suspensions, were injected intraperitioneally 4 and 24 h before ischemia. The anti-ischemia effect was assessed by the number of animals surviving more than 7 days. The results were subjected to statistical analysis by the Student and chi-square tests.

EXPERIMENTAL RESULTS

Estimation of the content of LPO products in the brain of the rats at various times after creation of total ischemia in the animals revealed an increase in all parameters studied (Table 1, Fig. 1a). The DC concentration in the first 5-30 min was 1.3-1.5 times higher than initially, and after 30 min of ischemia the increase was insignificant. The increase in concentrations of KD and MDA was significant starting from the 5th-15th minute of ischemia, and throughout the period of investigation thereafter The KD level was 1.8-2.5 times and

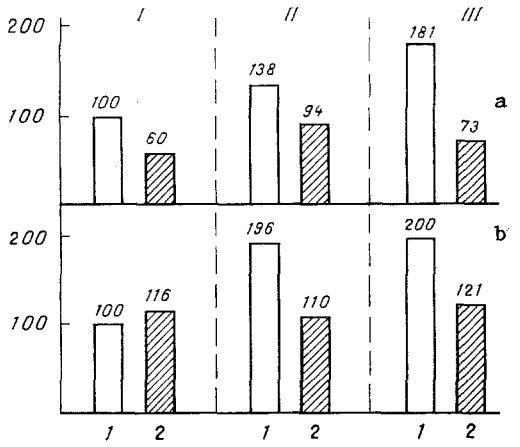

Fig. 2. Effect of ionol on level of LPO products (in % of initial level) in rat brain after ischemia (II) and reperfusion (III). I) Initial value. a) DC, b) MDA. 1) No antioxidant given, 2) injection of ionol (120 mg/kg 4 h before ischemia). Ischemia for 30 min, reperfusion for 20 min.

the MDA level 1.6-2 times higher than initially, and the maximal rise in the concentration of secondary LPO products was observed after 30-60 min of ischemia.

Reperfusion of the brain after ischemia for 30 min (Table 1; Fig. 1b) was accompanied by a significant increase in the DC concentration (after 20 min of reperfusion, to 133% of its level before reperfusion) and by a tendency for the concentrations of secondary LPO products to increase (MDA after 5 min, CD after 20 min). After reperfusion for 60 min the level of all LPO products investigated fell, close to their concentration in the ischemic brain without reperfusion, but the concentration of secondary products at this time was significantly higher than initially.

The results of estimation of survival of the animals after different periods of total cerebral ischemia and the results of a study of the protective action of the antioxidants are given in Table 2. Injection of ionol and diludin 4 h before creation of ischemia prolonged the period of cerebral ischemia which could be tolerated to 25-35 min compared with 15 min in the control; the number of animals surviving after 25 and 30 min of ischemia was higher, moreover, when both antioxidants were used than after 15 and 25 min of ischemia in the experiments without antioxidants. Comparison of the action of equal does of ionol and diludin (120 mg/kg), injected into the animals at the corresponding times (4 h before ischemia) revealed a higher anti-ischemic effect of ionol. The percentage of animals surviving after 25 min of ischemia was 82 and 33 ($p < 0.05$) and after 30 min 44 and 7 ($p < 0.05$), respectively, for ionol and diludin. Ionol had its strongest protective action in doses of 120 and 240 mg/kg, and when given 4 and 24 h before creation of ischemia, the effect was about equal.

The study of the concentrations of LPO products in the brain of rats receiving ionol by the optimal schedule (120 mg/kg 4 h before ischemia) showed (Fig. 2) that the compound completely inhibits DC and MDA accumulation in brain tissue both during 30 min of ischemia and after 20 min of reperfusion.

The results are thus evidence that total cerebral ischemia is accompanied by accumulation of primary and secondary LPO products. The concentration of primary products showed a smaller and less prolonged increase than the concentration of secondary products, which was discovered earlier in an investigation of ischemia of the limbs [3], and this is evidently due both to the rapid metabolism of primary LPO products in vivo and to exhaustion of readily oxidized substrates (and, perhaps, of oxygen) for their formation.

Comparison of the times of the initial increase in the level of LPO products in the brain with its increase in other organs [1, 2, 5] revealed the same order of precedence: brain (5 min) < liver (15 min) < heart (30 min) < kidney (60 min) < limb muscles (1-3 h), which is in the reverse order to the sensitivity of these organs to ischemia.

Reperfusion of the ischemic brain led to some activation of LPO processes, but under these circumstances the greatest increase was observed in the concentration of primary, and not of secondary, LPO products, perhaps due to the more intensive elution of secondary products than of primary, and in agreement with data on the increase in the concentration of easily soluble secondary LPO products (MDA) in the perfusate during reperfusion of the myocardium [12].

A high protective effect of the antioxidants ionol and diludin, previously noted for ischemia of other organs [4], also was discovered in the present investigation; the wide range of their possible application (4-24 h before creation of ischemia) was confirmed and, in addition, ionol was shown to have advantages over diludin.

The results are further evidence of the universal character of the role of LPO processes in the pathogenesis of injuries due to total ischemia and reperfusion of organs and they confirm the need for the search and optimization of methods of their prevention and treatment by antioxidants.

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