ANTIOXIDATIVE EFFECTS OF ANTIOXIDANTS IN CEREBRAL ISCHEMIA

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The key mechanism of brain damage in acute ischemia and, in particular, ischemia and subsequent recirculation, is activation of free-radical processes, leading to intensification of lipid peroxidation (LPO) in nerve cell membranes and to their destruction [1, 5, 14, 15]. Antioxidants can limit lipid peroxidation in brain tissue in acute ischemia, and this may be manifested as weakening of neurological disturbances and improved ability to tolerate ischemia [1, 5, 13].

Meanwhile the strongest protective effect under these conditions is given by preparations from the antioxidant group [4, 6]. Information on the ability of antihypoxants to limit LPO in stress and certain forms of hypoxia [3, 7] motivated us to assess the antioxidative properties of antihypoxants (sodium hydroxybutyrate, bemetil, etomerzol) in ischemia of varied duration and ischemia with recirculation, compared with those of the antioxidant emoxypine.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male and female Wistar rats weighing 200-250 g. Incomplete cerebral ischemia was induced by ligation of both carotid arteries under ether anesthesia. The animals were killed by decapitation, and their heads were immediately frozen in liquid nitrogen. The brain was then removed and ground in a porcelain mortar. Lipids were extracted by Folch's method and the lipid content in the chloroform extract was determined gravimetrically. The intensity of LPO was judged by the results of determination of conjugated dienes (CD) [16] and Schiff's bases (SB) [18]. The antiradical activity of the preparations was assessed by the reaction with the stable free radical α-diphenyl-α-picrylhydrazyl (DPPH) [10]. The preparations were injected intraperitoneally 1 h before the creation of cerebral ischemia: sodium hydroxybutyrate in a dose of 100 mg/kg, bemetil and etomerzol in a dose of 50 mg/kg, and emoxypine in a dose of 5 mg/kg. The results were subjected to statistical analysis by Student's and Wilcoxon's tests [2].

EXPERIMENTAL RESULTS

The study of the brain levels of LPO products after cerebral ischemia of varied duration revealed a regular increase of all the parameters studied (Table 1), in agreement with observations of other workers [1]. After ischemia for 30 min the CD and SB levels exceeded those in animals undergoing mock operations by 30 and 42% respectively ($p < 0.05$). If ischemia lasted 3 h, the CD level remained raised (by 43%) and significant accumulation of LPO in the products took place: the SB concentration rose by 2.6 times. A similar trend in the content of initial and end products of LPO in brain tissue during ischemia of varied duration can be explained by the rapid metabolism of primary readily oxidized substrates [1].

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TABLE 1. Effect of Cerebral Ischemia of Varied Duration (30 min and 3 h) and Ischemia (30 min) Followed by Recirculation (1 h) on Brain Levels of Conjugated Dienes and Schiff's Bases (M ± m)

Parameter	Animals undergoing mock operation	Ischemia (30 min)	Ischemia (3 h)
CD, OD_{232}/mg lipid	0,056±0,004	0,073±0,003*	0,080±0,007*
SB, units/mg lipid	0,036±0,003	0,051±0,004*	0,095±0,016*

Legend. *$p < 0.05$ compared with animals undergoing mock operations.

TABLE 2. Effect of Emoxypine, Bemetil, Etomerzol, and Sodium Hydroxybutyrate on Brain Tissue Levels of Conjugated Dienes and Schiff's Bases during Ischemia (I, 3 h) and a Combination of Ischemia (30 min) with Recirculation (in h) (I + R) (M ± m)

Parameter	Emoxypine		Sodium hydroxybutyrate		Bemetil	Etomerzol	
	I	I + R	I	I + R	I	I	I + R
CD, OD_{233} mg lipid	0,066±0,003	0,068±0,008*	0,042±0,002*,**	0,056±0,002	0,064±0,008	0,064±0,004*	0,074±0,003*
SB, units/mg mg lipid	0,065±0,007*	0,052±0,002*	0,033±0,001*,**	0,058±0,002*	0,057±0,005*	0,049±0,006*,**	0,042±0,001*,**

Legend. *$p < 0.05$ compared with corresponding pathology, **$p < 0.05$ compared with emoxypine.

Significant accumulation of LPO products took place when the blood flow in the organ was restored after 30 min of ischemia.

Recirculation (1 h) led to an additional (compared with 30 min of ischemia) increase of CD by 60% and a twofold increase of SB.

Incomplete cerebral ischemia in rats (ligation of both carotid arteries) is thus accompanied by marked activation of LPO, which progresses with an increase in the duration of ischemia and resumption of the blood flow in the organ.

All the substances tested restricted LPO activation to some degree or other, both during ischemia alone and during ischemia with recirculation (Table 2). Compared with the control, emoxypine lowered the concentrations of primary and secondary LPO products in the ischemic rat brain by 18 and 46%, and after ischemia with recirculation, by 50 and 52% respectively ($p < 0.05$). Bemetil, which inhibited accumulation of CD and SB in the brain during ischemia by 20 and 40% ($p < 0.05$) respectively, exhibited antioxidant activity similar to that of emoxypine. Etomerzol significantly lowered the CD and SB levels by 20 and 50% respectively during ischemia and by 48 and 62% during ischemia with recirculation. Sodium butyrate inhibited LPO processes in the brain most strongly. This compound prevented accumulation of LPO products during cerebral ischemia, but only after ischemia and recirculation was the SB concentration raised by 61% ($p < 0.05$).

Thus the antihypoxants tested exhibited an effect on LPO comparable with that of the antioxidant emoxypine on two models of ischemia. Moreover, with respect to antioxidative activity, in some cases the antihypoxants surpassed emoxypine: sodium hydroxybutyrate for the intensity of its effect of levels on primary and secondary LPO products during ischemia with recirculation, and etomerzol for its effect on the CD concentration in both types of ischemia.

To clarify the possible mechanism of the antioxidant action of these preparations their antiradical activity was studied. Emoxypine lowered the optical density of the DFPH solution in the course of incubation for 2 h by 0.65-0.7 unit.

Not all antihypoxants tested exhibited antiradical properties. In the absence of direct antiradical activity of the antihypoxants, their strongly expressed antioxidative properties may probably be manifested through their action on mechanisms leading to limitation of LPO in the brain. One key mechanism of the antioxidative action of antihypoxants may be the ability of the preparations to limit the fall of pO_2 in the brain tissue during circulatory hypoxia [8, 9, 12]. In turn, the depth of hypoxia determines the severity of acidosis in the brain tissue [17]. Considering, on the one hand, the ability of sodium hydroxybutyrate and etomerzol to limit significantly lactate accumulation in the brain and the development of tissue acidosis [9, 12], and on the other hand, the direct connection between acidification of the incubation medium of the brain mitochondria and activation of LPO in them [11], revealed by different models of circulatory hypoxia, it is very probable that antihypoxants can limit LPO during ischemia through manifestation of their antiacidotic effect.

While not exhibiting antiradical activity, the antioxidants sodium hydroxybutyrate, bemetil, and etomerzol, can thus substantially limit LPO processes in brain tissue in acute incomplete ischemia, and sodium hydroxybutryate and etomerzol can do the same in ischemia with recirculation. As regards the intensity of their antioxidative activity. these compounds are not inferior to the antioxidant emoxypine, and in some cases they actually surpass it, It seems rational to use antihypoxants to control lipid peroxidation in acute cerebral ischemia.

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