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ACTIVATION OF PEROXIDATION OF BRAIN LIPIDS DURING INTRACEREBRAL HEMORRHAGE

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KEY WORDS: lipid peroxidation, homorrhage into the brain.

If the impedance of the brain is recorded in conscious cats after intracerebral hemorrhage (ICH) an early decrease is found in the capacity of the brain tissue, preceding the development of edema and swelling of the cells of the cerebral parenchyma [5], reflecting a disturbance of integrity of the membranes. The presence of a phase of acute compression anoxia [6] at the time of hemorrhage suggests that lipid peroxidation (LPO), an important injurious factor for nerve cell membranes during ischemia–anoxia of brain tissue [9], is involved in the pathogenesis of ICH.

The aim of the present investigation was to study the state of LPO in the brain tissue of rats during the first hours and days after ICH.

EXPERIMENTAL METHODS

Experiments were carried out on 58 noninbred male albino rats weighing 250-300 g. To produce ICH in the rats, a hollow needle was inserted stereotaxically under pentobarbital anesthesia (60 mg/kg) into the internal capsule of the right hemisphere. A week after the operation the experimental animals received an injection of 0.15 ml of autologous blood in the course of 2 min. Animals with a needle implanted into the brain but without ICH served as the control. The rats were decapitated 1, 3, and 24 h after ICH, the brain was quickly removed, cooled, weighed, and homogenized. Lipids were extracted with a mixture (2:1) of chloroform and methanol, with tissue and extracting mixture in the ratio of 1:17 (w/v) [17]. Immediately before extraction, nitrogen was bubbled through the mixture to remove oxygen. The concentration of primary products of LPO – diene conjugates (DC) – was determined spectrophotometrically in the lipid phase [11]. Antiradical activity (ARA) of the lipids was investigated with the aid of the stable free radical α-diphenyl-α-picrylhydrazide (DPPH) [8]. The concentration of malonic dialdehyde (MDA), a secondary LPO product, in the brain tissue and the kinetics of its formation in vitro during incubation of brain homogenate with pro-oxidants were investigated as in [10]. Two LPO processes were studied: nonenzymic oxidation, the so-called ascorbate-dependent pathway (ADP), and enzymic, or the NADPH-dependent pathway (NDP) [2]. The incubation medium contained 100 mM KCl, 20 mM Tris-HCl (pH 7.4), 12 μM Mohr's salt, 0.2 mM $Na_2P_4O_7$, and 1 mg/ml of homogenate protein. To study nonenzymic LPO 0.2 mM ascorbate was added to the medium, whereas to investigate enzymic LPO 0.3 mM NADPH was added. Samples, each of 2 ml, of medium were taken after incubation for 5, 15, 30, and 60 min and mixed with 2 ml of 30% TCA. The MDA level in the samples was determined after the reaction with 2-thiobarbituric acid [2]. The MDA concentration was expressed in nanomoles/mg protein. The protein concentration was determined by the biuret method [4]. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test [1].

EXPERIMENTAL RESULTS

ICH led to progressive accumulation of DC and MDA in the rats' brain (Fig. 1). An increase in the DC concentration in the brain was found 3 h (by 21%), and it was even greater

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TABLE 1. Kinetics of MDA Formation (in nmoles/mg protein) in Rat Brain Homogenate during Nonenzymic (A) and Enzymic (B) LPO (M ± m, n = 7)

Experimental conditions	Time of investigation	Incubation time, min				
		0	5	15	30	60
A	I	0,52±0,05	1,62±0,09	3,12±0,18	7,12±0,38	9,49±0,42
	II	0,56±0,04	1,78±0,10	4,21±0,20*	8,11±0,35*	10,81±0,46*
	III	0,58±0,04	1,85±0,13	4,84±0,31*	8,77±0,43*	11,68±0,51*
	IV	0,71±0,04*	1,90±0,17	4,17±0,21*	9,64±0,48*	12,32±0,66*
B	I	0,56±0,04	1,75±0,12	3,42±0,22	7,77±0,29	10,32±0,58
	II	0,60±0,04	2,17±0,14*	5,19±0,34*	9,18±0,36*	11,29±0,49*
	III	0,64±0,05	2,11±0,17	4,96±0,33*	8,39±0,42	10,45±0,65
	IV	0,59±0,05	1,63±0,16	3,34±0,26	8,11±0,37	10,07±0,62

Legend. I) Control; II, III, and IV) 1, 3, and 24 h, respectively, after ICH. *P < 0.05 compared with control.

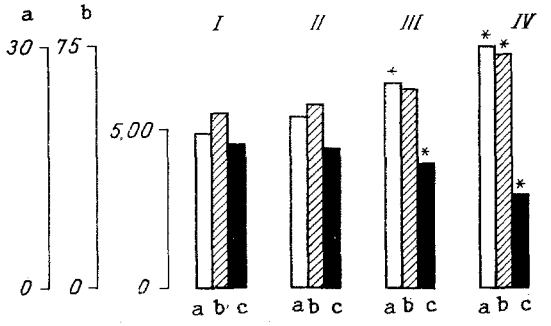

Fig. 1. Changes in LPO parameters in rat brain after ICH. a) DC (in optical density units at 232 nm per milligram lipids); b) MDA (in nmoles/mg protein); c) ARA (in μmoles/g lipids); I) control; II, III, and IV) 1, 3, and 24 h respectively after ICH. *p < 0.05 compared with control.

(by 61%) 24 h, after ICH. The increase in the MDA concentration became statistically significant after 24 h of the experiment. Changes in ARA of the lipids were opposite in character: a significant decrease after 3 and 24 h (by 16 and 37% respectively). A similar relationship between DC and ARA of the lipids is characteristic of various noxious influences acting on the body and leading to activation of LPO [3].

During ICH the combination of acute anoxia (in the phase of the escape of blood into the brain parenchyma) followed by a period of relative normoxia (soon after the end of intracerebral hemorrhage) [6] was highly favorable for activation of LPO, as has been demonstrated on models of circulatory anoxia [9]. Incidentally, 1 h after ICH there was no change in the DC and MDA concentration in the brain or in ARA of the brain tissue lipids. Meanwhile, investigation of the kinetics of MDA accumulation at this period revealed an increase in the ability of brain homogenates to carry out LPO (Table 1). Compared with the control (animals undergoing a mock operation) MDA accumulation in the brain homogenates of the experimental rats was accelerated. Under conditions of nonenzymic LPO, for instance, the rate of MDA accumulation in the rats exceeded that in the control by 14, 23, and 35%, 1, 3, and 24 h after ICH. This is evidence of the early formation of conditions favoring LPO activation in the brain tissue of rats with an intracerebral hematoma.

Distinct changes in DC and ARA were observed 3 and 24 h after ICH. At these times, the LPO initiators were probably joined by iron, released from hemoglobin as a result of hemolysis of the erythrocytes of the hematoma, for according to some data [10], deposition of hemosiderin in nerve cells is observed during the first few hours after intracerebral hemorrhage. The significant increase in DC and decrease in ARA of the nerve tissue lipids in the present experiments coincided in time with the accumulation of fluorescent LPO products after injection of iron chloride into the cortex [12]. In addition, 24 h after ICH circulatory anoxia of brain tissue arises [6], and can intensify LPO processes [9]. The combination of

circulatory anoxia and accumulation of iron in the brain parenchyma evidently leads to a further increase in concentrations of LPO products in the brain and a decrease in ARA of lipids.

The results of the present investigation thus demonstrate progressive activation of LPO in brain tissue in the acute period of intracerebral hemorrhage, and is the justification for the early use of antioxidant therapy.

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INTERACTION OF PENTACYCLIC PROGESTERONE DERIVATIVES WITH PROGESTIN-BINDING SITES OF UTERINE CYTOSOL

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Introduction of an additional D' carbon ring, condensed with the steroid skeleton in the 16α,17α-positions, into the progesterone molecule led to the creation of a new class of highly active progestogens [5], known as pregna-D'-pentarans. The activity of these compounds, tested in vivo on rabbits in the Clauberg–McPhail pregnancy supporting tests, was 3-9 times greater than the activity of progesterone itself [1]. X-ray structural analysis of compounds of the pentaran series showed virtual coincidence of the geometry of the A, B, and C rings with that of progesterone, and the existence of differences only in the conformation of the D ring and the side chain [2]. Meanwhile the pentarans which have been tested revealed completely unexpected extremely low relative affinity for progesterone receptors in the cytosol of the guinea pig uterus, between limits of 1 and 15% [7].

In the investigation described below interaction of progesterone and some of its derivatives with an additional D' carbon ring (I-X) with progestogen-binding systems of the uterine cytosol of the rabbit, rat, guinea pig, and man, was studied.

EXPERIMENTAL METHODS

A 10 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM EDTA and 10% glycerol was used; ^{3}H-progesterone was obtained from Amersham International (England) and ^{3}H-promegestone – synthe-

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