ACTION OF ETHANOL AND LIMONTAR DURING ANTENATAL DEVELOPMENT ON LIPID PEROXIDATION AND ON THE ANTIOXIDANT PROTECTION SYSTEM IN THE BRAIN AND LIVER TISSUE OF FETAL AND NEONATAL RATS

L. P. Bykova and T. P. Zhukova

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Activation of lipid peroxidation (LPO) is one manifestation of the action of ethanol [9, 12, 13] and leads to injury to biological membranes and disturbance of their permeability. To prevent the damaging action of the highly reactive products formed as a result of LPO, the cell possesses an enzyme system of antioxidant protection, which also reacts to ethanol intake [4, 10]. The damaging action of ethanol has been studied mainly in the adult, and in particular, in liver tissue. These processes have virtually not been studied in fetal and neonatal animals exposed antenatally to the action of ethanol. Knowledge of these processes is essential for the search for substances protecting the fetus against the toxic action of ethanol.

We have studied the antenatal action of ethanol on LPO and on the enzyme system of antioxidant protection in the brain and liver tissue of fetal and neonatal rats. The action of an alcohol protector (limontar), developed at the Laboratory of Tissue Metabolism Regulators, Research Institute for Biological Testing of Chemical Compounds, Ministry of the Medical and Microbiological Industry of the USSR, and made available to us for trials, was tested.

EXPERIMENTAL METHOD

Experiments were carried out on 44 pregnant Wistar rats, divided into four groups: the animals of group 1 (E) received an injection of 30% ethanol in a dose of 5 g/kg; those of group 2 (EL) received 30% ethanol in a dose of 5 g/kg preceded by limontar (1 mg/kg), those of group 3 (L) received limontar alone in a dose of 1 mg/kg, and those of group 4 (C) served as the control, and received water to drink. All preparations were injected by gastric tube from the 16th through the 19th days of pregnancy. Altogether 104 fetuses at the 20th day of intrauterine development and 77-day-old rats were investigated. The brain was quickly removed and frozen in liquid nitrogen, then homogenized for 1 min in 0.05 M potassium–phosphate buffer (pH 7.8) with 0.1 mM EDTA in a ratio (w/v) of 1:15. Liver homogenates were prepared in the same way in 0.05 M Tris-HCl buffer (pH 7.4) with 0.1 mM EDTA. The homogenates were centrifuged at 3500 rpm for 15 min. To determine LPO products and enzymes of antioxidant protection, the supernatant was used; in the case of enzymes the homogenates were incubated in the cold for 3-5 min with 0.1% Triton X-100 (final concentration). Lipids were extracted with a mixture of chloroform and methanol. The content of Schiff bases (SB) was measured in an extract of lipids on a "Hitachi" fluorometer by the method in [8], using a solution of quinine sulfate in 0.1 N sulfuric acid (1 mg/kg) as the standard; the concentration of SB was determined by the intensity of fluorescence (in % of the standard) calculated per milligram of total lipids. Conjugated dienes (CD) were determined at 233 nm by the method in [6], their concentration being expressed in optical units per milligram lipids, total lipids by a kit from "Lachema" (Czechoslovakia), the malonic dialdehyde (MDA) concentration spectrophotometrically in brain and liver homogenates on the basis of their content of products reacting with 2-thiobarbituric acid, by the method in [7]. The MDA level was calculated in nmoles/kg tissue.

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TABLE 1. Content of LPO Products in Brain and Liver Tissue of Fetal and Neonatal Rats Exposed to Experimental Factors during Last Third of Period of Intrauterine Development (M ± m)

Group	Fetus			Newborn rat		
	MDA, nmoles/mg tissue	CD, optical units/mg lipids	SB, % mg lipids	MDA, nmoles/mg tissue	CD, optical units/mg lipids	SB, % mg lipids
			Brain			
C	0,40±0,11 (11)	0,21±0,05 (10)	0,45±0,09 (10)	0,53±0,14 (8)	0,22±0,05 (3)	0,40±0,14 (6)
E	0,82±0,45** (14)	0,21±0,03 (11)	0,71±0,17*** (10)	0,73±0,18* (7)	0,36±0,05* (3)	0,36±0,11 (5)
EL	0,68±0,18* (5)	0,29±0,06** (5)	0,53±0,10^{+} (4)	0,72±0,15* (8)	0,29±0,07 (11)	0,48±0,06 (4)
L	0,49±0,14^{+} (7)	0,26±0,02^{++} (7)	0,45±0,02^{+++} (3)	0,63±0,15 (8)	0,20± ±0,02$^{\pm\pm}$,$^{\circ\circ}$ (8)	0,40±0,05$^{+,\circ\circ}$ (4)
			Liver			
C	0,40±0,14 (10)	0,22±0,05 (10)	1,22±0,36 (11)	1,22±0,13 (6)	0,27±0,05 (3)	1,34±0,28 (3)
E	0,42±0,13 (15)	0,19±0,04 (18)	1,31±0,27 (14)	1,65±0,75 (5)	0,27±0,05 (6)	0,14±0,0 (1)
EL	0,38±0,11 (6)	0,19±0,03 (9)	1,06±0,24 (7)	0,82±0,26** (8)	0,33±0,04 (5)	1,19±0,43 (5)
L	0,49±0,11 (6)	0,15±0,02 (6)	1,21±0,26 (6)	1,32±0,33° (7)	0,27±0,06 (6)	0,92±0,25 (5)

Legend. *p < 0.05, **p < 0.01, ***p < 0.001 compared with group C; +p < 0.05, ++p < 0.01, +++p < 0.001 compared with group E; $^{\circ}$p < 0.02, $^{\circ\circ}$p < 0.01 compared with group EL; number of animals shown in parentheses.

TABLE 2. Activity of Enzymes of Antioxidant Protection in Brain and Liver Tissues of Fetal and Neonatal Rats Exposed to Various Factors in Last Third of Period of Intrauterine Development (M ± m)

Group	Fetus				Newborn rat			
	GR	GP	SOD	catalse	GR	GP	SOD	catalase
	nmoles NADPH/mg protein/min		units of activity/mg protein		nmoles NADPH/mg protein/min		units of activity/mg protein	
				Brain				
C	11,4±1,6 (14)	9,0±1,7 (11)	6,5±2,7 (18)	0,76±0,16 (14)	7,4±1,7 (11)	10,2±1,5 (9)	7,6±7,9 (7)	0,62±0,11 (10)
E	11,4±1,8 (18)	9,7±1,7 (15)	5,0±2,7 (20)	0,84±0,14 (18)	8,5±1,3 (7)	13,8±5,3 (6)	7,1±1,9 (6)	0,69±0,18 (6)
EL	11,7±1,8 (12)	12,5±1,8^{+++} (11)	5,2±1,6 (14)	0,78±0,15 (13)	8,8±1;1* (8)	6,2±1,0*** (8)	6,6±0,5 (6)	0,71±0,10 (9)
L	11,9±1,4 (11)	13,7± 1,9***, $^{+++}$ (9)	5,6±1,2 (12)	0,78±0,14 (12)	9,4±1,9* (8)	10,2±2,2$^{\circ\circ\circ}$ (9)	5,9±1,4 (8)	0,74±0,15 (9)
				Liver				
C	29,4±4,3 (11)	42,7±9,2 (10)	7,1±2,7 (6)	12,47±4,33 (14)	24,1±4,9 (7)	50,0±5,7 (7)	6,8±2,7 (8)	9,70±3,04 (6)
E	25,8±3,0* (20)	48,4±5,9 (20)	5,3±0,7 (12)	9,10±2,43** (19)	27,3±8,1 (6)	40,5±6,9* (6)	5,4±1,9 (5)	9,17±3,20 (7)
EL	30,9±4,7^{+++} (14)	53,1±4,4**, $^{++}$ (14)	6,2±1,1^{++} (8)	12,24±3,35^{+++} (14)	28,1±7,0 (10)	47,1±16,5 (10)	7,5±1,2^{+} (10)	12,74±1,95*,$^{+}$ (11)
L	34,7±6,8^{+++} (12)	53,8±7,0**,$^{+}$ (12)	4,9±2,5 (9)	12,43±2,58^{++} (12)	29,7±6,3* (9)	42,0±10,6 (9)	7,2±1,2 (9)	13,28±2,49*,$^{+}$ (7)

Legend. *p < 0.02, **p < 0.01, ***p < 0.001 compared with group C; +p < 0.05, ++p < 0.01, +++p < 0.001 compared with group E; $^{\circ\circ\circ}$p < 0.001 compared with group EL; number of animals given between parentheses.

Activity of glutathione reductase (GR) and glutathione peroxidase (GP) was determined on a "Pye Unicam SP-800" spectrophotometer at 340 nm on the basis of the rate of oxidation of NADPH [1,11,15], and was expressed in micromoles of NADPH oxidized during 1 min. Catalase activity was determined as destruction of hydrogen peroxide at 240 nm [5],

taking as the unit of activity the difference between logarithms (to base 10) of hydrogen peroxide concentrations during 1 min. Superoxide dismutase (SOD) activity was measured by the method in [14] in the modification in [2] on the basis of inhibition of auto-oxidation of adrenalin. The quantity of SOD needed to inhibit the oxidation of adrenalin by 50% was taken as the unit of activity. Activity of all the enzymes was calcualted per milligram protein. The protein concentration was determined by the microbiuret method [3]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Antenatal exposure to ethanol caused activation of LPO in the brain tissue of the fetal and neonatal rats: the MDA level was raised by 53 and 27% respectively, the content of SB in the fetuses was increased by 37%, and the CD concentration in the newborn rats was increased by 39% (Table 1). Activity of enzymes of antioxidant protection in the brain tissue of these animals did not differ from the control values (Table 2).

By contrast with the brain, in the liver tissue of the animals of group E accumulation of LPO products did not take place (Table 1), but a decrease in activity of enzymes of antioxidant protection was found. In the fetuses, for instance, GR activity (by 20%) and catalase activity (by 27%) were reduced, although GP and SOD activity remained unchanged. Reduction of GP activity by 23% was observed in newborn rats; no change in the activity of the other enzymes was found.

Thus the mechanism of action of ethanol on the brain and liver tissue evidently differs. In the brain ethanol causes activation of LPO without any change in the antioxidant protection system, whereas in the liver, activity of several enzymes of this system is depressed.

Injection of limontar 40 min before ethanol led to normalization of some of the parameters of LPO in the brain tissue. In the EL group, for instance, the SB level in the fetuses and the CD level in the newborn rats fell to the control values, but the MDA concentration remained increased in both fetuses and newborn animals (Table 1). Activation of some enzymes of the antioxidant protection system due to the action of limontar was found in the brain tissue of animals of the EL group: in the fetuses GP activity was increased (by 29%), GR activity was increased (by 19%) in neonates (Table 2).

In the liver tissue limontar restored normal activity of enzymes of the antioxidant protection system, when depressed by ethanol (GP and catalase activity in the fetuses, GP in the newborn rats). GP activity in the fetuses and catalase activity in the day-old animals also exceeded the control values (Table 2).

Limontar had a similar effect on the enzyme system of antioxidant protection in intact animals of the L group (Table 2).

Thus limontar activates certain enzymes of antioxidant protection and thereby facilitates manifestation of the tissue protective reaction, so that it can be used as a protector against alcohol.

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