the accumulation of the DETC-Fe-NO complex was completely blocked by 2 mM ethylene glycol tetraacetate, a specific chelator of Ca^{2+} ions, which is an obligatory component of the NO-synthase reaction; 3) the accumulation of the DETC complex was inhibited by the specific inhibitors of NO-synthase N-methyl-L-arginine (by 70% at a concentration of 1 mM) and nitro-L-arginine (by 60% at a concentration of 1 mM).

Thus, the method proposed here may be used for correct determination of NO-synthase activity in dense brain homogenates. The method is also applicable for enzyme preparations of various purity (in these cases auxiliary problems concerning the incubation conditions and addition of Fe(II) should be experimentally solved), but in the case of dense homogenates and tissue samples, where the level of effectors regulating NO-synthase activity is maximally preserved, this method offers the greatest advantages.

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Antenatal Effects of Ethanol and Limontar on Lipid Peroxidation and the Antioxidant Defense System in the Brain and Liver of Rat Progeny

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In contrast to short-term exposure, prolonged exposure to ethanol in the antenatal period is found to inhibit lipid peroxidation in the brain and liver of rats. Activation of the system of antioxidant defense in the brain and liver is observed after both short- and long-term exposure to ethanol. After short-term ethanol exposure, limontar normalizes lipid peroxidation.

Key Words: *antenatal effect of ethanol; lipid peroxidation; enzymatic system of antioxidant defense; brain; liver*

Lipid peroxidation (LPO) activated by ethanol impairs biological membranes and their permeability [10,12]. The enzymatic system of antioxidant (AO) defense also responds to ethanol administration [5,13]. These processes are still to be researched in newborns exposed to ethanol before birth. Such studies are necessary in order to find ways of protecting the fetus from the toxic effect of ethanol.

Previously we investigated LPO and the activity of AO defense enzymes in the brain and liver of rat fetuses and newborns after a short-term exposure to ethanol [1].

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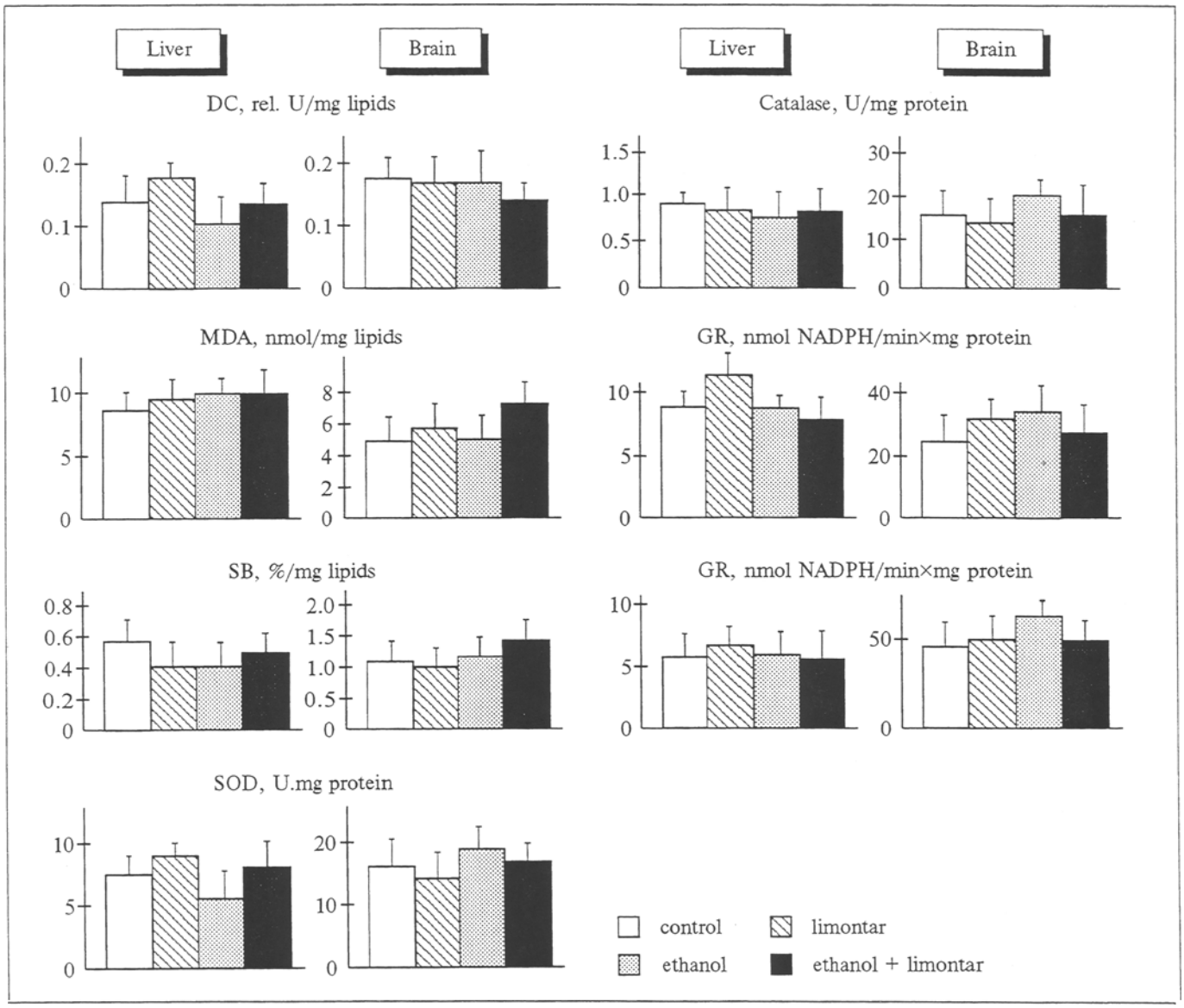

Fig. 1. Content of LPO products and activities of AO enzymes in the brain and liver of 7-day rats after experimental exposures in the last third of uterine development.

In the present study we compared the short- and long-term antenatal effects of ethanol on LPO and on the activity of AO enzymes in the brain and liver of rats by the end of the first week of life. As in our previous work, we tested the effects of limontar, an alcoprotector, whose main components are succinic and citric acids. This agent was produced at the Laboratory for Tissue Metabolism Regulators at the Research Institute for Biological Testing of Chemical Compounds and was given to us for trials.

MATERIALS AND METHODS

Fifty-two pregnant Wistar rats were used. The animals were divided into four groups: group 1 animals (E) were administered 30% ethanol in a dose of 5 g/kg, group 2 rats (EL) were given 30% ethanol in the same dose in parallel with limontar in a dose of 1 mg/kg, group 3 (L) were administered limontar in a dose of 1 mg/kg, and group 4 (C) were controls given water. All the preparations were administered intragastrically through a probe. Two experimental series were performed. In the first series all the named agents were administered starting from the 16th till the 19th day of gestation (a short-term exposure), and in the second starting from the first till the 20th day, that is, throughout the whole pregnancy (long-term exposure). Eighty-five 7-day-old rats were examined. The brain was rapidly removed and frozen in liquid nitrogen, and then homogenized for 1 min in

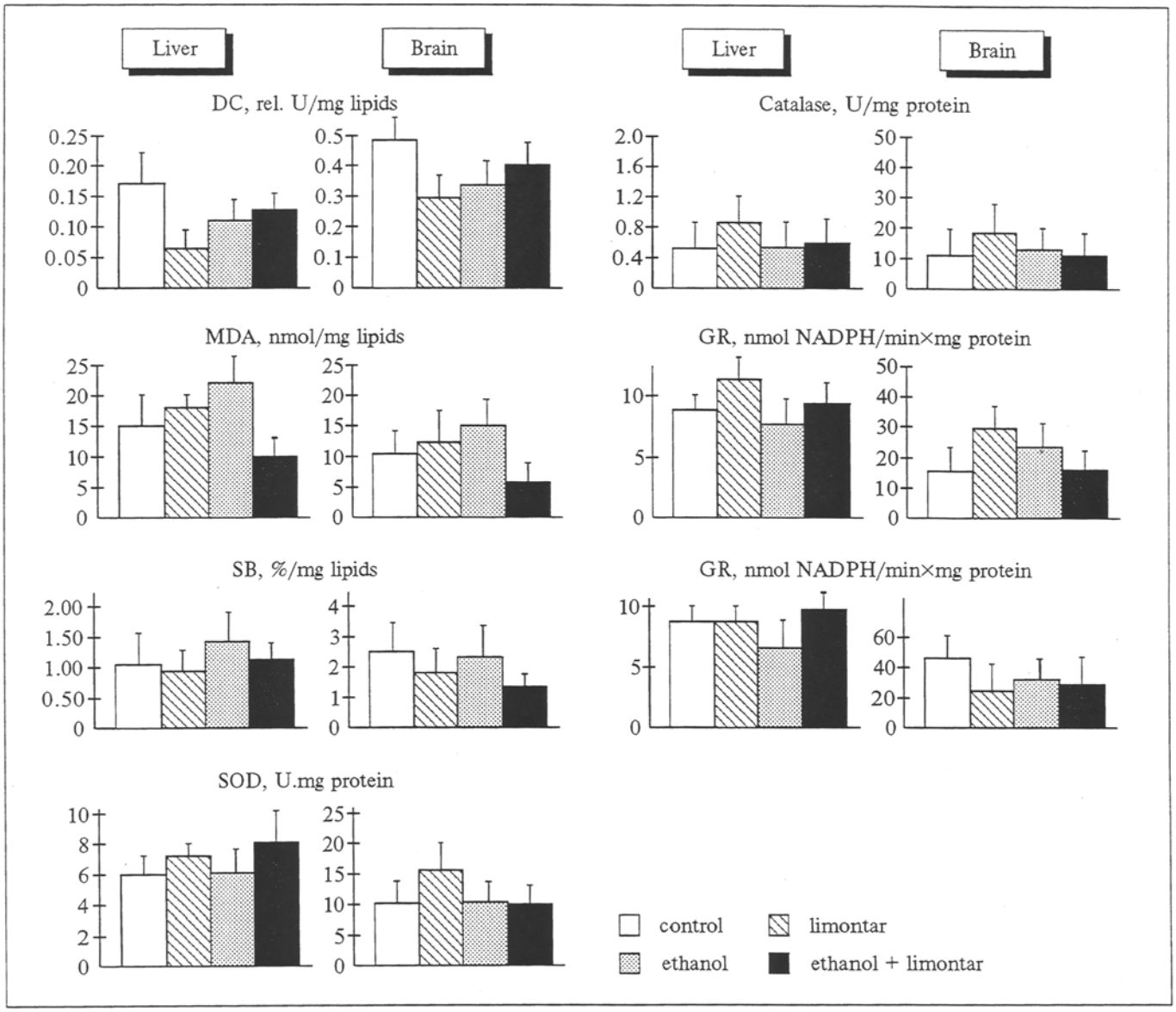

Fig. 2. Content of LPO products and activities of AO enzymes in the brain and liver of 7-day rats after experimental exposures over the entire course of intrauterine development.

0.05 M potassium phosphate buffer, pH 7.8, with 0.1 mM EDTA in a 1:15 ratio (mass:volume). Liver homogenates were prepared similarly using 0.05 M Tris-HCl, pH 7.4, with 0.1 mM EDTA. Brain and liver homogenates were centrifuged at 1000 g for 15 min. LPO products and AO enzymes were measured in supernatants; for enzymatic assays the homogenates were incubated in the cold for 3 to 5 min with 0.1% Triton X-100 (final concentration). Lipids were extracted with a chloroform-methanol mixture. Schiff's bases (SB) in lipid extracts were measured with a Hitachi fluorometer using a previously described method [9] with quinine sulfate solution in 0.1 M sulfuric acid (1 μg/ml) as the reference; the SB concentration was assessed by the intensity of fluorescence (in % of the reference) calculated per mg of total lipids. Diene conjugates were measured spectrophotometrically (233 nm) using a previously described method [7], and their concentration was expressed in optical units per mg of lipids, total lipids were measured using a Lachema (Czechoslovakia) kit; malonic dialdehyde (MDA) was determined spectrophotometrically at 535 nm in brain and liver homogenates by the levels in these homogenates of products reacting with 2-thiobarbituric acid using a previously described technique [8]. The MDA level was calculated in nmoles per mg of total lipids.

Superoxide dismutase (SOD) activity was measured by a previously described [14] modified [3] method according to the inhibition of adrenaline autooxidation. The amount of SOD required for

50% inhibition of the rate of adrenaline autooxidation was taken as an activity unit. Catalase activity was measured spectrophotometrically at 240 nm from hydrogen peroxide degradation [6], the difference between common logarithms of hydrogen peroxide concentrations in one minute taken as an activity unit. Glutathione reductase (GR) and glutathione peroxidase (GP) activities were measured with a Pue Unicam SP-800 spectrophotometer at 340 nm by the rate of NADPH oxidation in one minute. The activities of all enzymes were calculated per mg of protein. Protein concentration was determined by the microbiuret method [4]. The results were statistically processed using Student's *t* test.

RESULTS

In contrast to our previous results, when we detected LPO activation in 20-day fetuses and animals on the first day of life [1], no increase in LPO products was observed in the brain or liver of 7-day rats which had been exposed to ethanol on days 16-19 of intrauterine life, which may indicate a certain normalization of this process by the end of the first week of life (Fig. 1). The studied AO enzymes normalized in the progeny of rats by the end of the first week of life both in the brain (increase of SOD activity by 21% and of GR activity by 19%) and liver (a 31% increase of GR activity) after a short-term antenatal exposure to ethanol (Fig. 1). This appears to be a delayed adaptational response of the AO defense system to antenatal ethanol exposure, and it was probably responsible for the LPO normalization.

On the other hand, exposure of rats to ethanol during the entire period of antenatal development resulted in a reduction of some LPO characteristics in both the brain (the level of DC dropped by 61%) and liver (the DC level dropped by 42%, and the SB level by 29%) on day 7 of life (Fig. 2). Moreover, AO enzyme activation was revealed in 7-day animals after prolonged antenatal exposure to ethanol: in the brain GR activity was increased by 18% and SOD activity by 27%, while in the liver GR activity was increased by 44% and catalase activity by 41%, this being paralleled by a decrease of GP activity by 41%. The detected decline of GP activity could be due to a drop of the level of reduced glutathione under the influence of ethanol, which is known as this enzyme's cofactor [13].

Limontar caused an increase of AO enzyme activities, especially in liver tissue of 7-day-old rats after a short-term antenatal exposure, this indicating the presence of AO properties in limontar. A short-term limontar and ethanol exposure was conducive to normalization of the AO defense enzymes whose activities were increased under the effect of ethanol (Fig. 1).

Long-term exposure to limontar, on the other hand, led in the control animals to a reduction of LPO parameters (DC) and AO enzymes, this possibly being due to limontar's own AO properties. Prolonged combined antenatal exposure to ethanol and limontar resulted in a decrease of LPO parameters both in the brain (DC and MDA) and in the liver (DC, MDA, and SB) of 7-day rats, this decrease being attended by normalization of the activities of the majority of the AO defense system enzymes (Fig. 2).

Hence, after a short-term exposure to ethanol, limontar normalized the ethanol-activated LPO process due to the activation of AO enzymes and its own AO properties. The alcoprotector characteristics of limontar were less pronounced after a long-term exposure to ethanol. Nevertheless, our results permit us to recommend that limontar be included in combined therapy and in the prophylactic treatment of children born to mothers abusing alcohol.

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