CHANGES IN ACTIVITY OF ANTIOXIDATIVE ENZYMES AND LIPID PEROXIDATION LEVELS IN BRAIN TISSUE OF EMBRYOS EXPOSED PRENATALLY TO ETHANOL

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KEY WORDS: antioxidant system; lipid peroxidation; ethanol; embryos.

Although our ideas on the teratogenic and embryotoxic action of ethanol are long standing, the concrete mechanisms of this process have not yet been fully explained. Much information has now been gathered to show that many toxic substances (including ethanol) exert their action by stimulating the formation of toxic free-radical products of oxygen and by activating lipid peroxidation (LPO) Free-radical mechanisms are currently regarded as a universal damaging factor in a wide range of pathological conditions [1, 7]. On the basis of available data, a role for LPO in the hepatotoxic action of ethanol can be confidently ascribed [6, 10]. Data on the involvement of free-radical processes in the neurotoxic effect of ethanol are more contradictory, bearing in mind that damage to the nervous system resulting from the action of ethanol on the offspring, one of the characteristic features of its toxicity, and it was accordingly decided to study changes in LPO levels and activity of enzymes of the antioxidant system, namely catalase and superoxide dismutase (SOD), and the concentration of reduced glutathione (GlSH) in the brain of embryos exposed to maternal alcohol consumption.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rats weighing 200-250 g. From the first day of pregnancy until their use in the experiment, the animals were given 20% ethanol solution as the sole source of drinking fluid. The control animals received water The animals took part in the experiments after the 13th-17th day of pregnancy. The embryonic brain, after removal, was washed with 50 mM Na-phosphate buffer (pH 7.4), and homogenized in the same buffer in the ratio (w/v) of 1:4 or 1:6. The homogenate was centrifuged for 25 min at 4000*g*. The residue was discarded and the supernatant centrifuged again for 60 min at 15,000*g*. The final supernatant was collected and used for determination of SOD activity and the LPO level. The intermediate residue of the mitochondrial fraction was washed once with the above-mentioned buffer and homogenized in 1% Triton X-100 detergent (made up in the same buffer) in a volume equal to that of the original homogenate. The homogenate was centrifuged for 30 min at 15,000*g*. The supernatant was used to determine catalase activity.

SOD activity of the brain tissue was determined by a method based on inhibition, by the sample containing SOD, of oxidation of NADH by superoxide radicals [9]. Catalase activity was determined titrometrically [2]. The tissue LPO level was determined by measuring LPO products reacting with 2-thiobarbituric acid [4]. Activity of the enzymes and the LPO level were calculated per milligram soluble protein of the tissue extract. The concentration of GlSH was determined by measurement of its reaction product with alloxan [3].

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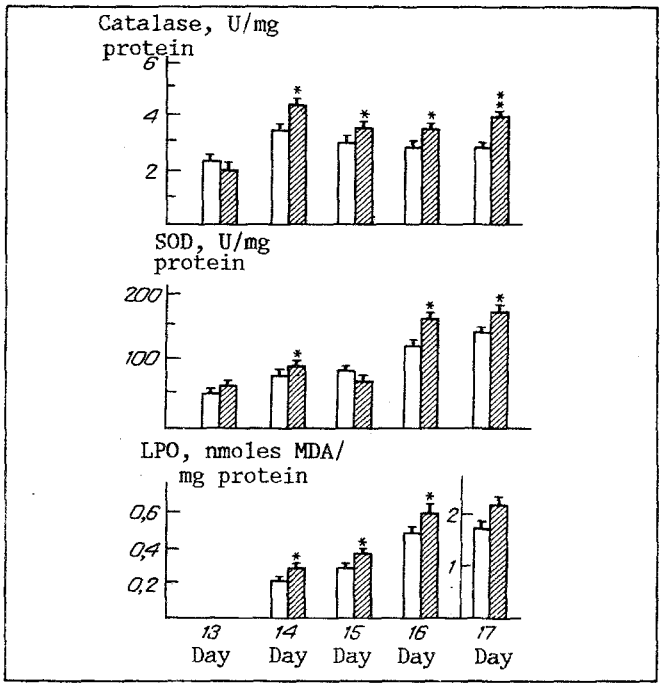

Fig. 1. Action of ethanol on catalase and SOD activity and on LPO level in brain of 13-17-day embryos. Unshaded columns – control; shaded – ethanol. *$p < 0.05$; **$p < 0.01$.

EXPERIMENTAL RESULTS

The experimental results showed that ethanol consumption by pregnant rats is accompanied by elevation of lipid peroxidation levels in the brain tissue of 14-17-day embryos by 10-20%, This increase was significant on the 14th-16th day (Fig. 1). This fact indicates activation of free-radical oxidation. Simultaneously with elevation of the LPO level in the embryonic brain, starting with the 14th day of intrauterine development catalase activity was increased by 15-30%, indirect evidence of more intensive formation of hydrogen peroxide, a toxic product of oxygen reduction, in the brain of the embryos. The results of the study of SOD activity in embryonic brain tissue in the course of intrauterine alcohol administration, indicate stimulation of the activity of this enzyme in the embryonic brain tissue toward the 14th day of embryogenesis by 15% (Fig 1). Toward the 16th and 17th days of embryogenesis the increase in activity was 40% and 25% respectively, the difference between them being significant. The rise of SOD activity can be connected with activation of superoxide radical generation and subsequent substrate induction of enzyme activity.

The experimental results thus show that the cause of activation of free-radical oxidation in the embryonic brain in alcohol poisoning is evidently increased formation of free-radical products of oxygen.

The results of numerous investigations show that the intracellular LPO level is controlled not only by antioxidative enzymes, but also by nonenzymic antioxidant systems, including reduced glutathione [5]. It was demonstrated previously that chronic alcohol poisoning leads to a fall in the GlSH concentration in liver tissue, and this is considered to be one cause of elevation of the LPO level in the liver [6, 8]. Our experiments showed that chronic alcohol consumption by pregnant rats leads to a reduction in the GlSH concentration in the brain tissue of 15-day embryos (32.1 ± 1.4 mg% in the control, 27.9 ± 0.8 mg% in the experiments, $p < 0.05$). It can accordingly be concluded that the cause of activation of free-radical oxidation in the embryonic brain tissue during chronic alcohol consumption by pregnant rats may be not only a direct increase in the formation of free-radical reduced products of oxygen, but also a decrease in power of the nonenzymic antioxidant system (GlSH), under conditions when activity of antioxidant enzymes is insufficient to restore the normal LPO level. It can thus be considered that activation of free-radical oxidation of lipids in embryonic brain tissue during exposure to ethanol may play a role in the neurotoxic action of alcohol on embryo and fetus.

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EFFECT OF A SYNTHETIC CHOLECYSTOKININ DERIVATIVE ON HORMONE SECRETION IN HUMAN FETAL PANCREATIC TISSUE CULTURE

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The gastrointestinal hormone cholecystokinin (CCK) participates in the regulation of pancreatic endocrine function. Experiments in vivo and in vitro have shown that CCK stimulates basal secretion of insulin and glucagon [4], alters the sensitivity of the β- and α-cells to glucose [5], and modifies the paracrine effects of insular hormones [6]. It has been suggested that CCK derivatives, by selectively stimulating insulin secretion, may be used in the clinical management of diabetes. Indian research workers have synthesized an analog of the C-terminal tetrapeptide CCP (CCK-4), namely pro-Met-Asp-Phe-NH_2 (PMAP). In concentrations of 10^{-10}-10^{-6} M, PMAP stimulated insulin secretion in a culture of rat islets just as effectively as native CCK-4 [2]. Meanwhile PMAP (but not CCK-4) did not stimulate glucagon secretion [3].

In the investigation described below the effect of PMAP on insulin and glucagon production was studied in cultures of microfragments (Mfr) of the human fetal pancreas.

EXPERIMENTAL METHOD

To prepare primary histotypical cultures the pancreas from three 20-week human fetuses was used. The method of culture and details of the morphology of the Mfr were described previously [1]. The Mfr were grown in dishes in medium 199 (glucose concentration 5.5 mM) with 10% fetal calf serum, 5 mM essential amino acids, and 5 mM of a vitamin mixture. The prepared Mfr from each pancreas (on the 5th-6th days in vitro) were divided into four groups and transferred in 24-well planchets. The Mfr of each group were distributed so that for each time of the experiment there were three wells, containing from 8 to 15 Mfr. Nfr of group 1 (control) were incubated in medium 199 with 2.5% serum, amino acids,

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