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CHANGES IN BRAIN LIPID PEROXIDATION IN THE FETAL ALCOHOL SYNDROME

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Since the clinical picture of the so-called fetal alcohol syndrome (FAS) was described [4] in 1968, it has been the subject of many clinical and experimental investigations. However, the picture of the neuropathological processes determining the central nervous correlates of the cognitive defects developing after perinatal exposure to ethanol, is still far from a full explanation. We know that ethanol passes readily through biological membranes and can thus affect the enzyme systems which maintain the ionic gradients through neuronal membranes. One factor which lies at the basis of the damaging action of alcohol is intensification of liquid peroxidation (LPO), induced by alcohol [8, 9], which leads to quantitative and qualitative changes in the lipid composition of the membranes. We accordingly decided to study to what degree perinatal exposure to alcohol is reflected in LPO processes in different regions of the animal brain.

EXPERIMENTAL METHOD

Experiments were carried out on male rats aged 12 weeks, divided into three groups, with 10 animals in each group. The 1st group consisted of young rats whose mothers had received a 6% aqueous solution of ethanol as the

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TABLE 1. Effect of Ethanol on Accumulation of Fluorescent LPO Products and Conjugated Dienes in Rat Brain Tissues (M ± m, n = 10)

Brain tissue	Experimental conditions	Fluorescent products, relative units, with 325 mg/mg of protein	Conjugated dienes, mmoles/mg protein
Cortex	Control	10,13±1,03	0,10±0,02
	Group 1	32,79±3,37*	0,26±0,03*
	Group 2	24,53±3,30*	0,14±0,02
Hippocampus	Control	21,50±1,88	0,19±0,03
	Group 1	72,49±5,15*	0,65±0,13*
	Group 2	77,32±9,92*	0,42±0,06*
Striatum	Control	22,62±2,10	0,43±0,05
	Group 1	54,48±5,10*	0,48±0,13
	Group 2	45,73±4,71*	0,46±0,07
Hypothalamus	Control	26,23±3,05	1,32±0,21
	Group 1	58,25±8,91*	0,21±0,04*
	Group 2	83,44±12,12*	0,61±0,12*
Cerebellum	Control	9,75±1,43	0,08±0,03
	Group 1	26,49±2,81*	0,05±0,02
	Group 2	30,90±3,69*	0,17±0,03*

Legend. Asterisk indicates values for which $p < 0.05$ compared with control.

sole source of fluid during pregnancy. The quantity of ethanol consumed during 24 h was about 9 g/kg body weight. The copulation index, the discovery of sperm in the vagina of a female sharing a cage with a male, and not previously having given birth, was taken as the first day of pregnancy [2]. Young rats born to-these mothers were fed by other foster rats, which had received the usual supply of water during pregnancy, but which had been given a 6% aqueous solution of ethanol instead of water during lactation (the cross fostering method).

Rats of the 2nd group were the offspring of mothers receiving the ordinary water supply during pregnancy, but fed after birth by foster rats, and which received 6% ethanol instead of water only during lactation (the cross fostering method).

The 3rd group consisted of control animals, born to and fed by mothers receiving sucrose solution, equal in calorific value to 6% ethanol, instead of water.

On reaching the age of 12 weeks (i.e., 9 weeks after the cessation of exposure of the experimental rats to ethanol) animals of the three groups were sacrificed and the frontal cortex, striatum, hypothalamus, hippocampus, and cerebellum were dissected from their brains, and whose tissues were used to prepare homogenates in K,Na-phosphate buffer, pH 7.4 (0-4°C), by means of a glass homogenizer with Teflon pestle at 1200 rpm for 3 min. The protein content of the homogenate was determined by the biuret method, and then adjusted to 20 mg/ml. To 1 ml of the homogenate thus prepared 6 ml of a chloroform:methanol mixture (ratio of chloroform to methanol v/v = 2:1) was added. The mixture was aerated for 1 min (at 18-20°C), then centrifuged at 4000*g* for 10 min. The bottom (chloroform) layer was transferred into a test tube, and in order to determine conjugated dienes, the chloroform was evaporated on a rotary vaporizer, and the dry residue was dissolved in n-hexane. The absorption spectrum of the sample was recorded at 233 nm. The intensity of fluorescence was determined in the chloroform phase during excitation at 360 nm and emission at 442 nm [1]. The spectrofluorometer (Perkin–Elmer 44B, USA) was calibrated beforehand with a solution of quinine sulfate.

EXPERIMENTAL RESULTS

Perinatal exposure to ethanol caused an increase in the content of fluorescent products, compared with the control animals, in all brain structures investigated, both in rats born to mothers receiving ethanol during pregnancy and lactation and in rats born to mothers receiving alcohol only during lactation (Table 1). A significant increase in the concentration of conjugated dienes relative to the control animals also was observed in the hippocampus of both groups of experimental animals. Conjugated dienes were increased in the cerebral cortex only in rats born to mothers

receiving alcohol during both pregnancy and lactation, whereas an increase in conjugated dienes in the cerebellum was observed in rats born to mothers receiving alcohol during lactation only.

The results show that alcohol consumption by pregnant and lactating mother rats is reflected in the steady-state concentration of LPO products in the brain of the progeny in the period of puberty (12-week-old rats).

Higher steady-state concentrations of fluorescent LPO products can be explained on the grounds that these are stable terminal products, which are very difficult to eliminate. As we know, these are complexes of lipoproteins which are components of the aging pigment – lipofuscin [3]. The results of model experiments indicate that the structure and molecular weight of the fluorescent LPO products are determined both by the degree of oxidation and by the composition of the cell lipids [3]. This explains both the differences in the intensity of LPO in different brain regions and the differences observed in the aftereffects of perinatal exposure to ethanol. For the marked increase in LPO products in the brain of rats with the FAS, a fact of essential importance is that the brain is exceptionally rich in polyunsaturated fatty acids, which are readily and rapidly oxidized.

The marked increase in steady-state concentrations of LPO products appearing sooner (conjugated dienes) shows that with time the aftereffects of activation of LPO subside, and in some parts of the brain (the hypothalamus in our experiments) may even go on to give a rebound effect – a fall of the LPO level below normal.

Another particular feature which we noted when studying the intensity of LPO in the two groups of rats with SAS enables both these dependences of the effect of ethanol on free-radical reactions to be derived from ontogeny of the brain. In the rat, unlike in man, the brain develops most rapidly in the 5-10 days after birth. It can be tentatively suggested that this period of ontogeny of the brain, depending, perhaps on increased oxygen consumption, is the most flexible for the onset of free-radical oxidative reactions, and for the generation of oxygen radicals and molecular peroxide products. This marked tendency toward generation of highly reactive LPO products could help us to understand why the effect of ethanol is limited to the period of feeding, i.e., the period of brain ontogeny which includes the days of its strongest development, and why it is sufficient to cause an increase in LPO products in the brain, closely similar in its intensity to the increase developing after exposure to ethanol throughout the period of pregnancy and lactation.

However, the cause of the facilitated generation and accumulation of LPO products in the neonatal period may be the result of a deficiency of antioxidant and other protective mechanisms against LPO. In this connection one should note the existence of data [10] which suggest that the diminishing efficiency, in the course of aging, of those mechanisms which are responsible for ridding the cells of LPO products, is of the utmost importance for lipofuscin accumulation in neurons, characteristic of old age. We found that not only behavioral features and, in particular, the impairment of memory [7], but also the electron-microscopic and immunocytochemical changes in the brain [5, 6], observed in rats with FAS, resemble in many respects changes characteristic of old age.

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