BIOPHYSICS AND BIOCHEMISTRY

Effects of Low-Dose Ethanol on Rat Progeny upon Inhalation Exposure during Gestation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 3, pp. 265-267, March, 1996
Original article submitted February 17, 1995

Effects of ethanol in low concentrations on the course of pregnancy in rats and the development and metabolic characteristics of their progeny are studied: the morphology and function of pathological shifts are assessed and the level of endogenous ethanol in the blood and acetylcholinesterase activity in erythrocyte membranes measured. Inhalation exposure to ethanol is found to retard the development of the progeny. The consumption of ethanol by the progeny in the experimental group was 1.5 times higher than in the control. The results indicate that even trace amounts of ethanol in the atmosphere are toxic for the organism.

Key Words: *antenatal alcoholization; endogenous ethanol; progeny*

Under certain industrial conditions, ethanol may be present in the air as vapor and enter the organism of workers via the respiratory tract [2]. When inhaled, ethanol bypasses the liver and is absorbed in the lungs, after which it is delivered to organs and tissues, and, during gestation, to the fetus, readily crossing the placenta [5]. This study was aimed at experimental investigation of the aftereffects of antenatal alcoholization of the progeny of white rats with ethanol vapors in a dose which is considered safe for human beings.

MATERIALS AND METHODS

Experiments were carried out on outbred white rats of different age and sex - the progeny of mothers exposed to ethanol over the entire course of pregnancy.

After pregnancy was diagnosed, the females were placed in a chamber in which the concentration of ethanol was maintained at a level corresponding to the maximal permissible concentration for the work zone of industrial premises: 1000 mg/m^3 [2], monitored by gas chromatography.

Pregnant females were kept in the chamber 5 days a week for 4 hours daily (experimental group). To level the effect of an enclosure, females of the control group were placed in a chamber with ventilated air.

Morphological and functional parameters (body weight and size and parameters of physical development) were examined in newborn rats.

Endogenous ethanol (EE) was measured in the progeny after birth and at the age of 2 and 4 weeks and 2 and 3 months. Starting from the age of 2 weeks, blood for measuring the EE level was collected regularly in the same rats. The concentration of EE was measured by gas chromatography [9].

The progeny of control (n=40) and experimental (n=66) animals was divided into groups showing a preference for a 5% ethanol solution or water under conditions of free choice [6] after two selection tests.

After preference selection, the specific activity of acetylcholinesterase (ACE, EC 3.1.1.7) was measured in erythrocyte membranes [12] obtained by a previously described method [11].

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0007-4888/96/0003-0244$15.00

TABLE 1. Content of Endogenous Ethanol (μmol/liter) in the Blood of the Progeny of Control and Experimental Rats in the Course of Development

Group	Period of observation, months				
	0	0.5	1	2	3
Control	32.9±4.11	8.9±3.16	20.6±4.71	45.3±7.64	41.9±7.06
Experiment	55.9±4.11*	22.9±2.35*	19.0±4.74*	20.0±5.14*	27.6±5.88*

Note. Here and in Table 2: *$p<0.05$ in comparison with the control.

RESULTS

Antenatal alcoholization of females with ethanol vapors retarded the development of fetuses. Newborn control rats weighed 2.44±0.02 g, while the experimental neonates weighed 2.34±0.02 g ($p<0.05$). Body length of the newborns was also reliably lower ($p<0.05$) in the experimental group *vs.* the control: 2.92±0.009 *vs.* 2.95±0.008 cm, respectively.

In parallel with this, a high incidence of gross morphological abnormalities, such as hernias of different types, subcutaneous hemorrhages, and abnormal skeletal development, was observed in the progeny of the experimental group in comparison with the controls; moreover, the postnatal mortality was higher and the life span shorter in this group.

It is noteworthy that the reproductive function was impaired in the adult progeny of alcoholized females: more than 20% of females in the progeny never became pregnant, and in 35% pregnancy occurred only after repeated crossings. Other sequelae of antenatal alcoholization can be expected to include persistent metabolic disorders [4], and therefore we examined the time course of EE in the blood and the state of erythrocyte membranes in adult progeny.

The level of EE was found to be reliably higher in the blood of newborn rats (aged 1 day) of the experimental group. After two weeks its content fell in both the experimental and control groups (Table 1).

At the age of 1 month a "reversion" of the level of EE in the blood is observed, and later on it is in the control group that the EE level in the blood is reliably higher than in the experimental one. Adult progeny of control rats (both males and females) had higher levels of EE than animals of the experimental group (Table 2).

A low level of EE in the blood of antenatally alcoholized progeny may be regarded as a metabolic disorder and a risk factor for the development of alcohol preference. Previously a negative correlation was demonstrated between the EE level in the blood and ethanol consumption under conditions of free choice [3,7]. Hence, the next stage of our studies was to elucidate the attitude of adult progeny of alcoholized rats to ethanol. The preference index was calculated as the ratio of the amount of ethanol solution drunk to the total amount of liquid consumed during selection. The preference index was then divided by the body weight.

The ratio of the preference index to body weight was found to be reliably higher in antenatally alcoholized rats in comparison with the control: 3.55±0.23 and 2.62±0.21, respectively ($p<0.01$). It is worth noting that an individual positive attitude (preference index/kg) is also more frequent (more than 2-fold) in the experimental group. According to this criterion, about 60% of the experimental progeny may be counted as ethanol preferrers, whereas in the control group there were no more than 15 to 20% of such animals.

Ethanol, by acting upon the cell membranes, alters the arrangement of protein molecules and their lipid environment [10]. The effect reflects the cell tolerance and depends on the dose of ethanol and the sensitivity of animals to it [10,13]. Previously we demonstrated an increase of the specific activity of ACE in the striatal synaptosomal membranes of rats preferring ethanol [9]. Erythrocyte ACE is a membrane-bound enzyme capable of detecting the functional status of membranes to a certain extent [1].

Two weeks after selection the specific activity of erythrocyte membrane ACE was measured in the control and experimental rats. No reliable changes were detected: in the experimental group the activity was 15.6±1.87, in the control 12.7±1.34 nmol thiocholine× ×mg protein^{-1}×min^{-1}.

The results permit us to conclude that:

– inhalation of ethanol vapors during pregnancy leads to morphofunctional disorders in the development of the progeny;

– adult progeny of female rats alcoholized with ethanol vapors have a lower content of EE in comparison with intact animals;

– the type of alcoholization studied here is a hazardous risk factor for the development of ethanol preference to water in the progeny.

TABLE 2. Content of Endogenous Ethanol (μmol/liter) in the Blood of Adult Progeny of Different Sexes

Group	Males	Females
Control	37.8±2.15	49.5±5.20
Experiment	27.6±1.51*	29.1±4.28

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Accessory Groups of Nonapeptidergic Neurosecretory Cells of the Hypothalamus and Adjacent Regions of the Brain in Rats under Conditions of Dehydration

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 3, pp. 268-270, March, 1996
Original article submitted August 11, 1994

Study of the hypothalamus and adjacent areas of the brain in adult rats revealed that, along with the osmosensitivity of neurosecretory cells, the transport of vasopressin and oxytocin along their axons and their release from the terminals into the bloodstream are impeded. This is due to the absence of axonal bonds between the accessory groups of neurosecretory cells and the posterior lobe of the pituitary.

Key Words: *hypothalamus; accessory groups; vasopressin; oxytocin; dehydration*

The hypothalamus and adjacent areas of the brain in rats contain a number of accessory groups (AG) in addition to the chief nonapeptidergic neurosecretory centers (supraoptic, postoptic, and paraventricular nuclei) [14]. At present, the neuroanatomy of these formations has been described in detail [5], but the functional specialization of AG is not quite clear. Our previous experiments (immobilization and cooling) permitted us to hypothesize that some AG are involved in the regulation of the functions of the peripheral endocrine glands: thyroid and adrenal [4].

The task of this study was to investigate the histophysiology of AG under conditions of dehydration, which is a specific exposure for the entire nonapeptidergic hypothalamopituitary neurosecretory system. The status of the nonapeptidergic neurosecretory cells (NSC) - the principal nonapeptidergic formations in dehydration - has been studied frequently [7,9,10,12]. In contrast, the contribution of AG to the regulation of water-salt metabolism is still unclear.

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0007-4888/96/0003-0246$15.00