Brain Disorders in Fetal Alcohol Syndrome

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Intrauterine effect of alcohol on the development of cytomorphological structure of CNS in rats was studied by heterogeneous enzyme-linked immunosorbent assay. The level of transforming growth factor-β1 (TGF-β1) in animals during pregnancy was analyzed. Pronounced damaging effect of alcohol on brain cell in the progeny of alcoholized animals was demonstrated: loosening of nerve cells and degenerative changes in the form of pyknosis and chromatolysis in the cortex, hypothalamus, and cerebellum; subtotal decrease (sometimes complete absence) of neuroendocrine granules. The level of TGF-β1 was significantly increased in alcoholized pregnant females, which can attest to defects of the receptor apparatus of the target cells in both females and the progeny. Thus, the observed peculiarities of TGF-β1 expression are comparable to morphological changes in the brain and can be extrapolated to similar processes in humans (fetal alcohol syndrome).

Key Words: *children; effects of alcohol on the fetus; growth factors; fetal alcohol syndrome*

Abnormal pregnancy and delivery often have more detrimental effects on human nervous system and mental status than endo- and exocrine factors in the postnatal period. Along with somatic and infectious diseases in pregnant women, toxic exposures, including alcohol intake, occupy a special place among factors disturbing the normal course of pregnancy and affecting the fetal development both during pregnancy (small brain size and developmental defects) and after birth. Full-scaled clinical picture in the fetus induced by untrauterine alcohol exposure was called fetal alcohol syndrome (FAS) [2]. The diagnostics of FAS is now based on documenting of three facial signs (smooth philtrum, thin upper lip border, and short palpebral fissure), detection of body weight and height deficit and structural and functional abnormalities of CNS, and on the history of maternal alcohol abuse during pregnancy [1].

Most children with FAS are characterized by physical and mental retardation, hyperexcitation, and hyperactivity. They have reduced muscular tone, sleep disturbances, and impaired concentration and attention. They can successfully use stereotyped phrases in their speech, but are characterized by poor results in studies, delayed development, motor, cognitive, and speech habits, difficulties in transition from one activity to another, and impaired instinct of self-preservation (*i.e.* they cannot foresee the results of their actions). Poor motor coordination can lead to traumas. Children with FAS can be highly tolerant to pain. At school age they continue to grow slowly and have difficulties in communication. According to published data, FAS is the main cause of mental retardation [5].

Here we studied intrauterine effect of alcohol on the development of cytomorphological structure of CNS in experimental animals.

MATERIALS AND METHODS

The experiments were carried out in fall-winter period on 26 Wistar rats aging 1-2 months and weighing 280-300 g. The rats were maintained at room temperature under conditions of free access to food and water. The animals were divided into 2 groups: experimental (13 rats receiving 15% ethanol solution instead of water 1 month before mating and during pregnancy) and

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control (13 intact rats). For light microscopy, the brain obtained from 11 pups from each group was placed in neutral 5% formalin (at least 1:10 volume ratio). After fixation, blocks of the brain matter from the convexital sensorimotor cortex, hypothalamic area, and cerebellum were embedded in paraffin and stained with hematoxylin and eosin, after van Gieson, and after Nissl.

During pregnancy (days 9-12), the level of transforming growth factor-β1 (TGF-β1) was measured by heterogeneous enzyme-linked immunosorbent assay (Bender MedSystems test system) on a STAT FAX photometer.

The data were processed statistically by the method of alternative variation using Student's *t* test.

RESULTS

Histological examination of the brain from control animals revealed no appreciable deviations from normal. In the cortex, clear-cut stratification of nerve cells was preserved and slight pervascular edema in the form of inconstant dilatation of the Virchov–Roden–Snesarev space was seen. This phenomenon can be considered as a result of acute hypoxia related to animal euthanasia. In nuclei of hypothalamic area, nerve cells were evenly distributed, light secretory granules in their cytoplasm attested to preserved neuroendocrine function. In the cerebellar cortex, the granular layer was clearly seen, basket neurons (Purkinje cells) formed a layer at the boundary between the granular layer and white matter. They had round cytoplasm; nucleus and cytoplasm were clearly seen.

In some experimental animals, abnormalities of eyes, ears, and skull were visually seen. The cortex had foci of nerve cell loosening of different size and severity (Fig. 1). These foci were primarily localized in medium cell layers, but solitary loosening foci involved all layers. We also found degenerative changes in neurons, primarily in the form of chromatolysis in the medium layers and pyknosis in the surface layers.

In hypothalamic nuclei, loosening of nerve cells and degenerative changes in them were also seen (Fig. 2). Hyperchromatosis and pyknotic changes predominated, subtotal decrease (sometimes complete absence) of neuroendocrine granules was noted.

Cerebellar gyri were thinned. Strips of cell loosening and a sharp decrease in the number of basket cells were seen in the granular layer (Fig. 3). Preserved cells were unequally distributed at different levels and were characterized by hyperchromatosis and pyknotic changes.

When comparing the data of autopsy with the level of TGF-β1, we found an increase in TGF-β1 concentration in the experimental group to 187.9 ng/ml (*vs*. 129.7 ng/ml in the control; t=2.68, p<0.02). It is known that TGF-β1 participates in the regulation of cell growth, differentiation, and apoptosis starting from fertilization. Acculation of TGF-β1 in the blood can be related to ethanol blockade of TGF-β1 receptors in developing cells, which disturbs cell growth and leads to apoptosis [3].

It is also known that alcohol induces premature transformation of radial astroglia into astrocytes, thus disturbing migration of young neurons to their destination in the brain [4].

Our experiments demonstrated considerable damaging effect of alcohol on brain cells of the progeny of alcoholized animals: loosening of nerve cells and degenerative changes in the form of pyknosis and chromatolysis in the cortex, hypothalamus, and cerebel-

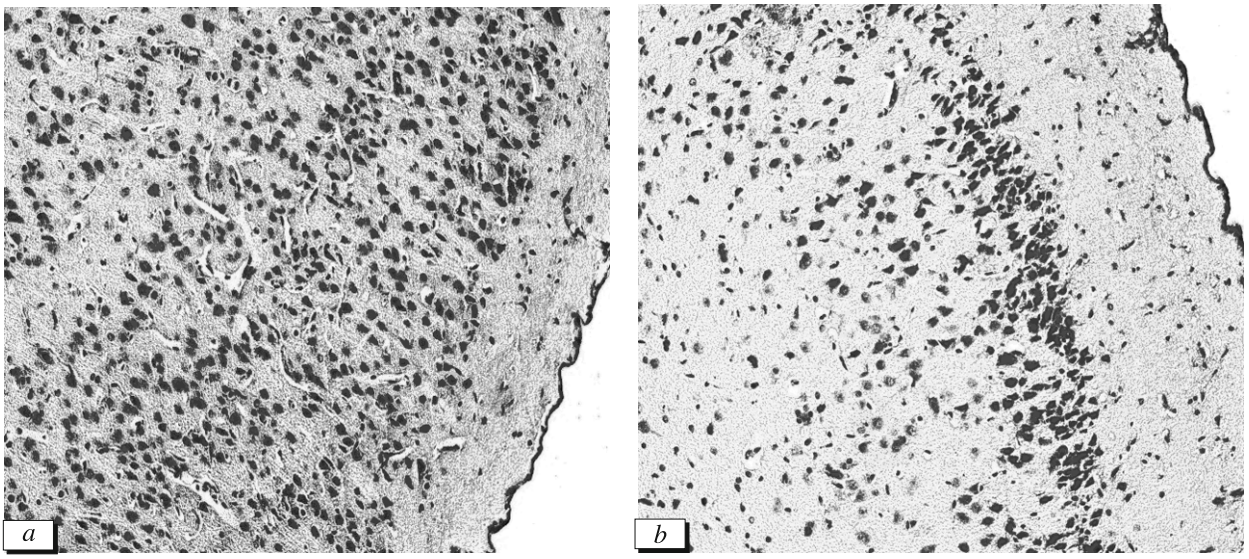

Fig. 1. Morphological characteristics of cerebral cortex. Here and in Fig. 2, 3: *a*) control group; *b*) experimental group. Hematoxylin and eosin staining.

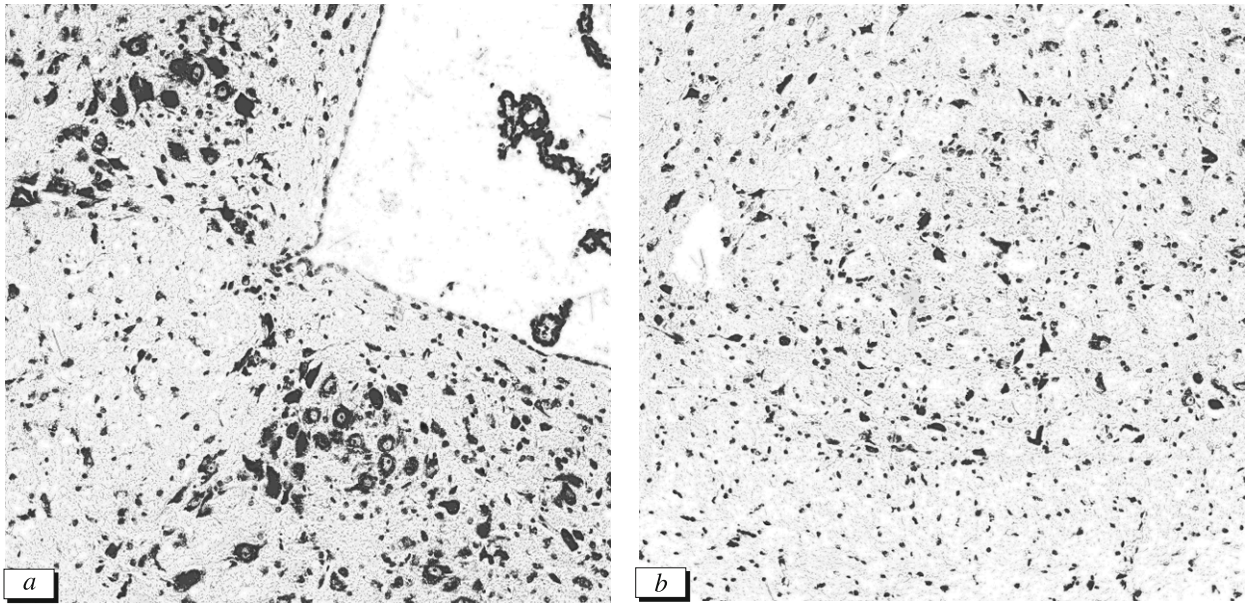

Fig. 2. Morphological characteristics of hypothalamic nuclei.

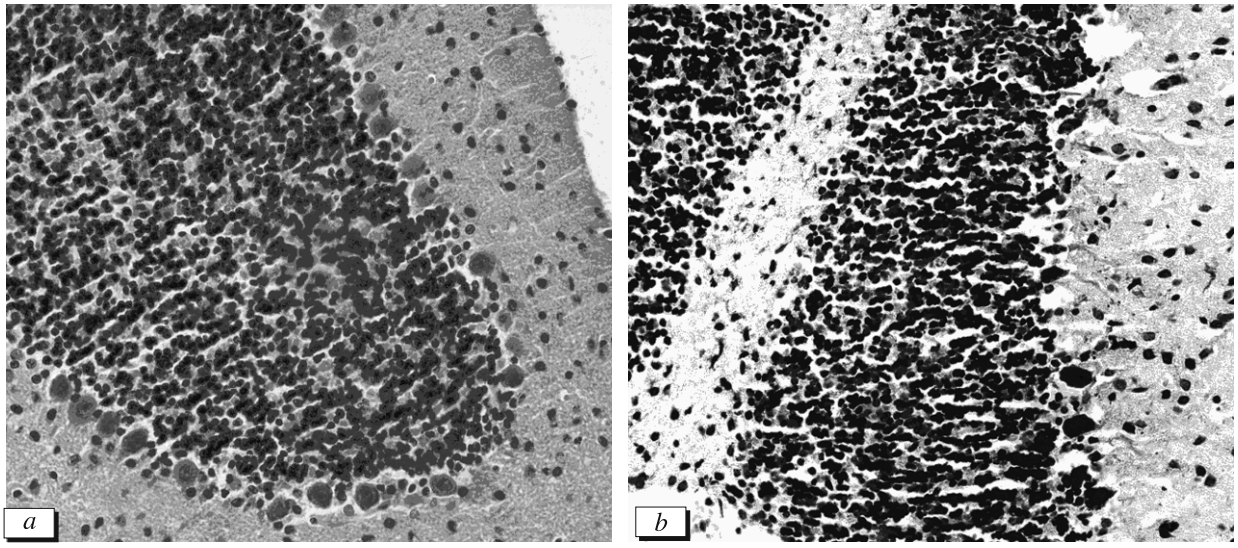

Fig. 3. Morphological characteristics of cerebellum.

lum; subtotal decrease (sometimes complete absence) of neuroendocrine granules.

The level of TGF-β1 was significantly increased in alcoholized pregnant females, which can attest to defects of the receptor apparatus of the target cells in both females and the progeny.

Thus, the observed peculiarities of TGF-β1 expression agree with morphological changes in the brain and can be extrapolated to similar processes in humans (fetal alcohol syndrome).

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