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Original article

Persistent neocortical astrogliosis in adult wistar rats following prenatal ethanol exposure

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Abstract

Timed pregnant wistar rats were divided randomly into groups A and B ($n=6$) each and C ($n=4$). Group A received a daily ethanol dose of 5.8 g/kg body weight per day, at 16.00 h on days 9–12th of gestation by intragastric intubations. Group B was pair-fed along with the treated rats and received an isocaloric solution of sucrose to substitute for the ethanol in the experimental group, for the same duration, while group C received standard chow and water ad libitum. The adult offsprings at 42 days of age, ($n=10$) from each group were sacrificed by whole body perfusion-fixation, after anaesthesia by an overdose of penthotal intraperitoneally. Specimens of neocortical samples were processed routinely for paraffin embedding and sections of 6 μm thickness stained for neurohistology. Another set of specimens was cryosectioned at −23 °C after cryoprotection in 30% sucrose/PBS and evaluated for GFAP immunohistochemistry. The study showed a distortion of the microanatomy of the neocortex in the treatment group A, particularly of layer V pyramidal neurons, which revealed mostly pyknotic pyramidal neurons with broken dendrites, collapsed cell bodies, obliterated nuclei and nucleoli. No differences were found between the brains from rats in groups B and C. There were widespread focal areas of reactive astrogliosis, more prominent within the layer V. Astrocytes demonstrated highly stained GFAP-positive immunoreactivity with heavy fibrillary processes in the neocortex of group A offsprings compared to the controls. The sub-pial regions were, however, sparse. In conclusion, this study confirms the hypothesis that microanatomical and microchemical changes following prenatal ethanol exposure persist into adulthood in rats.

Keywords: Astrogliosis; Cerebral cortex; Pia mater; Astrocytes; Adult rats; Fetal alcohol syndrome; Birth defects

1. Introduction

Prenatal exposure to alcohol is one of the leading preventable causes of birth defects, mental retardation, and neurodevelopmental disorders [1]. In 1973, a cluster of birth defects resulting from prenatal alcohol exposure was recognized as a clinical entity called fetal alcohol syndrome [2]. One of the most severe consequences of maternal ethanol consumption is the damage to the developing central nervous system [3–5], which is manifested by long-term cognitive and behavioral deficits in the offspring [6–10]. Prenatal exposure to ethanol affects many crucial neurochemical and cellular components of the developing brain [11–16] Alterations in astroglia development and in neuronal–glial interactions may also influence the development of the nervous system [17].

Glial cells constitute one of the most common cell types in the brain. They play critical roles in central nervous system (CNS) development. It has been demonstrated that glial cells are profoundly affected by prenatal alcohol exposure [11,18,19], suggesting that alterations in these cells may participate in CNS abnormalities associated with ethanol-induced teratogenesis [18,19]. In vivo studies show that prenatal exposure to alcohol hampers myelinogenesis and is associated with neuroglial heterotopias and abnormal astrogliogenesis [20]. Studies using primary cultures of rat cortical astrocytes show that ethanol affects DNA, RNA, and protein synthesis [21], decreases the number of mitotic cells [22], alters the content and distribution of several cytoskeletal proteins including the astroglial marker, glial fibrillary acidic protein (GFAP), and the levels of plasma-membrane glycoproteins [21]. It also reduces the capacity of

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astrocytes to secrete growth factors, and induces oxidative stress [23]. Furthermore, ethanol exposure during early embryogenesis alters the normal development of radial glia cells (the main astrocytic precursors) [11], delays the onset of GFAP expression, and decreases GFAP mRNA levels in fetal and postnatal brains and in radial glia and astrocytes in primary culture [24]. It has also been suggested that ethanol interferes with the transcription process of GFAP, thus leading to a reduction in GFAP-gene expression during astrogliogenesis [25]. However, brief exposure of rats to high levels of ethanol during the neonatal period (the period of astrocyte differentiation) causes a transient gliosis, with an increase in GFAP and its mRNA levels [18]. These findings indicate that astroglial cells are the important target of ethanol toxicity during central nervous system (CNS) development. This study reports new findings on astrogliosis in the adult wistar rat brain, prenatally exposed to ethanol.

2. Materials and methods

Adult female wistar rats (average weight 200 g) were used for the study. The rats were maintained in the Animal Holding of the Department of Anatomy and Cell Biology on standard mouse chow (Ladokun Feeds, Ibadan, Nigeria) and water ad libitum. The experiment conformed to guidelines on animal care and use currently applied in Nigeria. Mating was done by placing a virile male rat into a cage with two females at 16.00 h. The female rats were examined at 10.00 h the following morning for the presence of vaginal plug or spermatozoa, which was taken as gestation day 0 [26]. Following confirmation of pregnancy, the rats were divided randomly into two groups ($n=6$). Group A received a daily ethanol dose of 5.8 g/kg body weight per day, at 16.00 h on days 9–12th of gestation by intragastric intubations and group B received an isocaloric solution of sucrose which substituted the ethanol in the experimental group for the same duration. To control for the possible effects of undernutrition, rats in group B were pair-fed with ethanol-treated dams. An additional control group C ($n=4$), which were not intubated or pair-fed, but received standard chow and water ad libitum, was also included.

At age of 42 days the adult offsprings of either sex ($n=10$), from each group were anaesthesized by an overdose of penthotal intraperitoneally. The animals were whole-body perfused, first with normal saline, followed by 10% formol calcium fixative, under gravity. The whole brains were removed and a 6 mm coronal sample of the frontoparietal cortex (para central sulcus), was taken from each hemisphere under stereoscopic dissection, which were further fixed in the same fixative for 4 h. Specimens of samples were processed routinely for paraffin embedding and sections of 6 μm thickness cut on a sliding microtome (Histoslide 2000, Hamburg, Germany). They were left overnight at 37 °C in the incubator (for good adherence to the frost slides). The rest were cryosectioned (Fibrigol cryocut) at −23 °C, after cryoprotection in 30% sucrose/PBS. Thirty micrometer thick sections were then cut and stored in wells containing 1 ml of acidic phosphate buffered saline (PBS) at 2–8 °C, before immunohistochemistry.

Slides of paraffin sections were dewaxed and rehydrated according to standard protocol and stained in 2% crystal violet solution (Nissl stain) for 5–10 min, rinsed briefly in distilled water and then dehydrated with ascending grades of alcohol, cleared in xylene and mounted using Canada balsam and cover slipped.

2.1. Immunohistochemistry

Sections for GFAP immunohistochemistry were washed twice in Tris buffered saline (TBS, pH 7.4) at room temperature (RT) for 5 min, and endogenous peroxidase removed with 0.6% H_2O_2/1×TBS (pH 7.4) at RT for 30 min. Sections were washed thrice for 5 min in 1×TBS, RT, then equilibrated and blocked with 5% normal goat serum (NGS)/1×TBS for 60 min, and incubated in primary antibody (polyclonal rabbit-anti-GFAP 1:200, Gibco, Hamburg) at RT, for 60 min, and then washed thrice for 5 min each in 1×TBS at RT. Sections were then incubated in secondary antibody (goat-anti-rabbit-antiserum 1:50) at RT, for 60 min, washed thrice for 5 min each in 1×TBS and then incubated in rabbit-peroxidase-anti-peroxidase (PAP) 1:500 in TBS at RT, for 60 min, and then washed thrice for 5 min each in freshly prepared TBS (pH 7.6). Binding sites were visualized by incubating for the diaminobenzidine (DAB) reaction for 3 min at RT. The reaction was stopped by applying phosphate buffered saline (PBS, pH 7.4) to floating sections, and these were rinsed in distilled water. The sections were mounted on glycerin coated slides, air-dried overnight, and cover slipped with Entellan.

All sections were examined using the Axioplan-2 research microscope (Carl Zeiss, Germany) with a Sony 3-CCD video camera (Tokyo, Japan) connected to a Compaq computer that has the AxioVision 3.0-Rel software for digital image analysis. Photomicrographs were produced from the grabbed frames and printed out on glossy print paper using an Epson Photo stylus printer (Toronto, Canada).

3. Results

3.1. Nissl stain

Sections from the control group showed regular lamina formation of the cerebral cortex in the motor area with very prominent layer V pyramidal cells. These neurons were evenly distributed within the layer with distinct discernable apical dendrites (Fig. 1a). A typical pyramidal neuron (Fig. 1c) revealed a large centrally located pale round or