Experimental Neurology 229 (2011) 364–371

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Effects of prenatal ethanol exposure on rat brain radial glia and neuroblast migration

María Paula Aronne, Tamara Guadagnoli, Paula Fontanet, Sergio Gustavo Evrard, Alicia Brusco *

Instituto de Biología Celular y Neurociencias "Prof. Eduardo De Robertis", Facultad de Medicina, Universidad de Buenos Aires (UBA), Paraguay 2155 3rd fl., (C1121ABG) Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 15 March 2010
Revised 4 January 2011
Accepted 7 March 2011
Available online 14 March 2011

Keywords:
Prenatal ethanol exposure
Pax6
Neuronal migration
Corticogenesis
Cortical dysplasia
Fetal alcohol syndrome

ABSTRACT

Prenatal ethanol exposure (PEE) induces morphologic and functional alterations in the developing central nervous system. The orderly migration of neuroblasts is a key process in the development of a layered structure such as the cerebral cortex (CC). From initial stages of corticogenesis, the transcription factor *Pax6* is intensely expressed in neuroepithelial and radial glia cells (RGCs) and is involved in continual regulation of cell surface properties responsible for both cellular identity and radial migration. In the present work, one month before mating, during pregnancy and lactation, a group of female Wistar rats were fed a liquid diet with 5.9% (w/w) ethanol (EtOH), rendering moderate blood EtOH concentrations. Maternal gestational weight progression and fetal CC thickness were measured. CC from E12-P3 rats were examined for expression of vimentin, nestin, S-100b, Pax6 and doublecortin using immunohistochemical assays. RGCs expressing vimentin, nestin, S-100b and Pax6 had abnormal morphologies. The migration distance through the CC and the number of doublecortin-ir neuroblasts in germinative zones were decreased. We found significant morphologic defects on RGCs, a marked delay in neuronal migration, decreased numbers of neuroblasts, and decreased numbers of Pax6-ir cells in the CC as a consequence of exposure to ethanol during development. These observations suggest a sequence of toxic events that contribute to cortical dysplasia in offspring exposed to EtOH during gestation.

Introduction

Fetal alcohol syndrome (FAS) represents a public health issue associated with maternal alcohol abuse during pregnancy. Central nervous system (CNS) developmental defects, which can lead to microcephaly and mental retardation, are among the most significant characteristics of FAS. Different types of cortical dysplasia occur after prenatal ethanol exposure (PEE) due to, among other factors, disorders in neuroblasts migration (Miller, 1986). Although maturation of the cerebral cortex (CC) is completed during postnatal period, most CC gross architecture is established prenatally and crucially involves the orderly migration of neuroblasts from the proliferative ventricular zone (VZ), at the inner surface of the telencephalon, through the overlying intermediate zone to the cortical plate (Angevine and Sidman, 1961; Rakic, 1974). Neuroblasts, the precursors of neurons, migrate mainly attached to the cellular processes of radial glia cells (RGCs) by using them as living scaffolding.

During corticogenesis, RGCs perform a dual function: they behave as precursor cells (Choi and Lapham, 1978; Levitt et al., 1981, 1983; Misson et al., 1991) and as migratory substrates for neuroblasts (Rakic, 1972; Gadisseux et al., 1990; Hatten and Mason, 1990). RGCs are ultrastructurally similar to glial cells, e.g., they show bundles of filaments and glycogen accumulation (Rakic, 1972). They express nestin, a cytoskeletal type VI intermediate filament commonly used as a marker for immature cells in the developing CNS (Alberts et al., 2007; Hockfield and McKay, 1985); they also express vimentin, another cytoskeletal protein, a type III intermediate filament (Alberts et al., 2007; Pixley and DeVellis, 1984). Moreover, by the end of neurogenesis and during neuroblast migration, RGCs differentiate into astrocytes in some regions such as the CC (Pixley and DeVellis, 1984; Voigt, 1989). Upon differentiation into astrocytes, RGCs stop expressing vimentin and upregulate glial fibrillary acidic protein (GFAP; another type III intermediate filament) with an overlapping period when they express both (Bignami and Dahl, 1974; Levitt et al., 1983; Voigt, 1989). As mature astrocytes, RGCs also express S-100b protein, a dimeric neurotrophic and neurite outgrowth-promoting protein with many important actions during both pre- and postnatal life. Among these multiple functions, S-100b acts as a promoter of cytoskeletal stabilization (Donato, 2003; Donato et al., 2009; Huttunen et al., 2000). The correct specification of RGCs is therefore essential for normal corticogenesis (Pinto-Lord et al., 1982).

Pax6, a paired box family transcription factor, plays an important role in development of the brain and other organs (Chi and Epstein, 2002; Buckingham and Relaix, 2007). Pax6 is specifically expressed by RGCs and controls their differentiation in the CC (Götz, 1998; Götz et al, 1998; Simpson and Price, 2002). In

* Corresponding author. Fax: +54 11 5950 9626.
E-mail address: hbrusco@fmed.uba.ar (A. Brusco).

0014-4886/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2011.03.002

the absence of a functional Pax6, cortical RGCs exhibit altered morphology, cell number and cell-cycle kinetics (Götz et al, 1998). We previously showed that high blood ethanol concentrations (BEC between 119 and 300 mg/dl) alter Pax6 expression during prenatal cortical development (Aronne et al., 2008).

Neuroblasts migrating through RGC processes express doublecortin (DCX), a type of microtubule-associated protein (MAP) that regulates microtubule stability and influences the cytoskeleton dynamic state (Francis et al., 1999; Horesh et al., 1999). Doublecortin is widely expressed in migrating neuroblasts during prenatal and postnatal development in the CNS and during adult neurogenesis (Gleeson et al., 1999).

The cytoskeleton of nerve tissue cells is substantially altered by PEE (see Evrard and Brusco, 2011 for a comprehensive review). We showed that cytoskeletal proteins in neurons and glia are changed by exposure to both low (Ramos et al., 2002; Evrard et al., 2003, 2006) and high EtOH concentrations (Aronne et al., 2008). This current study examines the consequences of moderate BECs on Pax6 expression, RGCs and migratory neuroblasts in the developing CC.

Materials and methods

Animals were fed with a Liquid Diet LD 102 and LD 102A (PMI Alcohol Rodent Liquid Diet, USA). Ethanol (EtOH) (Sorialco S.A.C.I.F., Buenos Aires, Argentina) administered to the animals was analytical grade. For the study of the nuclear morphology we used Hoechst 33,342 stain (Sigma, St. Louis, Mo). Primary antibodies were: (a) mouse monoclonal vimentin (lot 071H4837, Sigma); (b) mouse monoclonal S-100b (lot 086H4864, Sigma); (c) rabbit polyclonal Pax6 (lot 0608036825; Chemicon International, Temecula, Ca); (d) guinea pig polyclonal doublecortin (DCX; lot 0605030194; Chemicon) and (e) mouse monoclonal nestin (lot 0704058033, Chemicon). Secondary antibodies were (a) goat anti-rabbit biotin-conjugate (lot 078H9160; Sigma); (b) goat anti-mouse-biotin conjugate (lot 078H9060; Sigma); (c) Texas Red anti-rabbit (lot T1002; Vector Laboratories, Burlingame, CA); and (d) Fluorescein anti-guinea pig (lot MFCD 00164607, Sigma). An avidin-peroxidase complex (Extravidin-Peroxidase®, lot 103K4840, Sigma), 3,3′-diaminobenzidine tetrahydrochloride (lot 65H0170, Sigma), nickel ammonium sulfate (Carlo Erba Reagenti; Milano, Italy) and hydrogen peroxide (H_2O_2; Merck KgaA; Darmstadt, Germany) were used for immunoperoxidase technique. All other chemical substances used in the experiments were analytical grade.

Animal treatment

Fifty adult nulliparous female Wistar rats (*Rattus norvegicus*; Hsd: WI) initially weighing 250–300 g were mated with 10 adult Wistar males. One month before mating (metabolic and taste acclimatization period) during pregnancy and lactation a group of females assigned to the treated group (T) were fed with the LD 102A liquid diet supplemented with 5.9% (w/w) EtOH. The control group (C) was fed the LD 102 control liquid diet. LD 102A powder is mixed with EtOH or maltodextrin to normalize calories between groups. All animals were kept in the same environment throughout the experimental period: 12/12 h light–dark cycle (lights off at 06:00 PM), controlled relative humidity (50–70%) and temperature (20 ± 2 °C), well-ventilated room and free access to liquid diets. Females were housed in groups of five and weighed each morning. Gestational day 0 (G0) was determined when sperm were found in vaginal smears, and thereafter the pregnant rats were housed singly, and assigned to five groups ($n = 10$/group). The five groups were: collection of E12, E14, E16 and E18 or P3 offspring.

The animal care for this experimental protocol was in accordance with the NIH guidelines for the Care and Use of Laboratory Animals and the principles presented in the Guidelines for the Use of Animals in Neuroscience Research by the Society for Neuroscience and with the CICUAL Animal Protocol of the School of Medicine (University of Buenos Aires, UBA).

Cesarean operation and specimen preparation

Pregnant rats were deeply anesthetized with ketamine (10 mg/kg) and xylazine (75 mg/kg) intaperitoneally. Uteri were extracted and immediately submerged in cold saline. Embryos were extracted in their membranes. Simultaneously, blood was collected in heparinized tubes from the maternal heart for determination of BEC. Heads of embryos were submerged in cold 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer (PBS) pH 7.4 for 4 h at 4 °C. The fixed heads were dissected and isolated from the surrounding skull tissues under a Wild Heerbrugg M5 stereomicroscope. Each brain was then submerged in 5% w/v sucrose in 0.1 M PBS, pH 7.4 for 12–24 h and then in a cryoprotective solution (25% sucrose w/v in 0.1 M PBS, pH

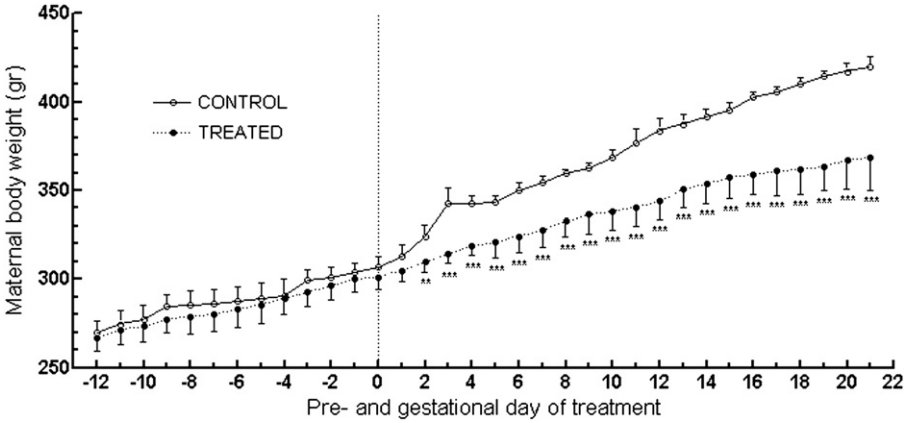

Fig. 1. Maternal weight gain showed that EtOH-exposed rats gained significantly less weight during gestation, starting at day 2, than control rats. Graph symbols represent mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.0001$.