Annals of Anatomy 200 (2015) 66–72

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Research article

Immunoreactivity of neurogenic factor in the guinea pig brain after prenatal hypoxia

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ARTICLE INFO

Article history:
Received 18 August 2014
Received in revised form 16 January 2015
Accepted 3 February 2015

Keywords:
Chronic placental insufficiency (CPI)
Prenatal hypoxia
Guinea pig
Neurogenesis

ABSTRACT

Chronic prenatal hypoxia is considered to cause perinatal brain injury. It can result in neurological disorders such as cerebral palsy or learning disabilities. These neurological problems are related to chronic placental insufficiency (CPI), which leads to chronic hypoxemia and hypoglycemia. The effects of hypoxia on neurogenesis during development have been a matter of controversy. We therefore investigated the effect of chronic prenatal hypoxia in the brain of the fetal guinea pig using the guinea pig CPI model. Chronic placental insufficiency was induced by unilateral uterine artery ligation at 30–32 days of gestation (dg: with term defined as ~67 dg). At 50 and 60 dg, fetuses were sacrificed and assigned to either the growth-restricted (GR) or control (no ligation) group. Immunohistochemistry was performed with HIF-1α, PCNA, NeuN and BDNF antibodies in the cerebral cortex and dentate gyrus. The number of NeuN-IR and BDNF-IR cells was lesser in GR fetuses than in controls in the cerebral cortex and dentate gyrus at 60 dg ($p < 0.05$). The growth of the developing brain is dependent upon the availability of growth factors such as BDNF. The reduction in the number of neuronal cells observed in our GR group was associated with the observed reduction in BDNF protein found at 60 dg. There was no significant difference between control and GR fetuses in the densities of PCNA-IR cells in the subventricular zone and subgranular zone at 50 and 60 dg. These findings suggest that the survival of neurons in the cerebral cortex is decreased by chronic prenatal hypoxia at 60 dg.

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1. Introduction

Chronic placental insufficiency (CPI), which leads to chronic hypoxemia and hypoglycemia (Jensen et al., 1996) causes intrauterine growth retardation (IUGR) which leads to abnormal prenatal development and neurological sequelae such as cerebral palsy, mental retardation, learning disability, and epilepsy (Towfighi et al., 1997; Tan et al., 1998; Berger and Garnier, 1999). Brain damage as a result of hypoxic injury during the fetal period is recognized as a major factor contributing to neonatal mortality (Towbin, 1970).

There has been some disagreement as to the effect of hypoxia on neurogenesis, which is the process of generating new neurons from progenitor cells including the proliferation, migration, differentiation and survival of neural precursor cells (Caviness et al., 1995). For example, progenitors within the neonatal mouse subventricular zone (SVZ) are vulnerable to chronic hypoxic/ischemic insult (Tolsa et al., 2004). However, chronic perinatal hypoxia promotes cell proliferation in the SVZ (Fagel et al., 2006). Neurogenesis is thought to be limited to the embryonic period, with the exceptions of the olfactory bulb, SVZ, and hippocampal dentate gyrus (Lichtenwalner and Parent, 2006). However, it has not known whether these neurons survive in the brain.

HIF1α is a nuclear protein complex that binds to a specific consensus sequence in hypoxia-responsive enhancers of many target genes (Semenza et al., 1994), the expression of which is induced by hypoxia-ischemia (Li et al., 2006) but it is rapidly degraded during normoxia (Wang et al., 1995). Therefore, we investigated HIF1α immunoreactivity to demonstrate that GR fetuses had been in a hypoxic condition.

Brain-derived neurotrophic factor (BDNF) promotes the growth and survival of dentate granule cells (Lowenstein and Arsenault, 1996) and pyramidal neurons (Ip et al., 1993) in the brain. The effects of chronic damage on BDNF expression are not well known. It has been shown that CPI reduces BDNF and tropomyosin receptor kinase B (Trk B) expression in the guinea pig hippocampus via reduced process outgrowth, while not affecting BDNF and Trk B expression in the cerebellum (Dieni and Rees, 2005). Furthermore,

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http://dx.doi.org/10.1016/j.aanat.2015.02.003
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the hippocampal volume is found to be reduced at term (Mallard et al., 1999) and hippocampal cell dendrites are altered (Dieni and Rees, 2003) in a guinea pig model of CPI, in which fetal hypoxia is induced via uterine artery ligation.

A wide range of opinions have been expressed on the effect of hypoxia in neurogenesis. However it has not well-known whether mature neurons survive in the brain. The present study used the guinea pig CPI model to investigate the effect of hypoxia on the development of mature neurons and to evaluate the relationship between BDNF and neurogenesis after chronic prenatal hypoxia.

2. Materials and methods

2.1. Animal surgery

All animal experiments were approved by the Chosun University Institutional Animal Care and Use Committee (approval no. CIACUC2013-S0009). CPI was induced via unilateral uterine-artery ligation in pregnant Dunkin–Hartley guinea pigs, as described previously (Nitsos and Rees, 1990; Mallard et al., 2000).Briefly, the animals were anaesthetized by intramuscular injection of Zoletil (10 mg/kg; Virbac, France) and xylazine (0.15 mg/kg; Bayer, Germany) at 30–32 dg (term ~ 67 days). After shaving the surgical area, a midline incision was made below the umbilicus under aseptic conditions. The fat pad of the uterine horns, which contained the maternal blood vessels, was exposed and ligated with silk sutures (4/0) at the cervical end of the arterial cascade. After the procedure, the abdomen was disinfected using povidone–iodine solution. The animals were caged and raised in a common environment.

2.2. Tissue preparation

The fetuses were delivered by cesarean section at 50 or 60 dg. Fetuses from the unoperated horn were assigned to the control group (50 dg, $n = 8$; 60 dg, $n = 8$), and those from the other, ligated horn were assigned to the growth-restricted (GR) group (50 dg, $n = 8$; 60 dg, $n = 8$). The fetuses were removed from the uterine horn and fixed in 4% paraformaldehyde (PFA) solution. The fetal brains were removed and transferred to fresh 4% PFA at 4 °C for 2 days. The forebrains were then washed with water, dehydrated through graded ethanol solutions, and finally embedded in paraffin. Series of 12-μm-thick sagittal sections were cut and mounted on gelatin-coated slides (Fisher Scientific, USA).

2.3. Immunohistochemistry

The deparaffinized sections were rinsed three times in 0.1 M phosphate-buffered saline (PBS; pH 7.4) and then incubated in 0.01 M sodium citrate buffer (pH 6.0) while being heated in a microwave oven for 10 min. After cooling, the slides were treated with 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase activity, and rinsed in PBS. They were then incubated with one of the following primary antibodies overnight at 4 °C: Rabbit anti-hypoxia-induced factor 1α (HIF1α; 1:500, Abcam, UK), monoclonal anti-proliferating-cell nuclear antigen (PCNA; 1:3000, Sigma, USA), mouse anti-hexaribonucleotide-binding protein-3 (NeuN; 1:100, Millipore, USA), and rabbit anti-BDNF (1:50, Santa Cruz Biotechnology, USA). On the following day the sections were washed several times in PBS and the immunoreactivity was visualized with biotinylated anti-mouse and anti-rabbit IgG and the avidin–biotin–peroxidase (ABC) detection system (Vectastain ABC Elite Kit, Vector Laboratories, USA), and chromogen 3,3′-diamino-benzidine as the chromogen. The sections were counterstained with thionine and coverslipped using PolyMount mounting medium (Polysciences, USA).

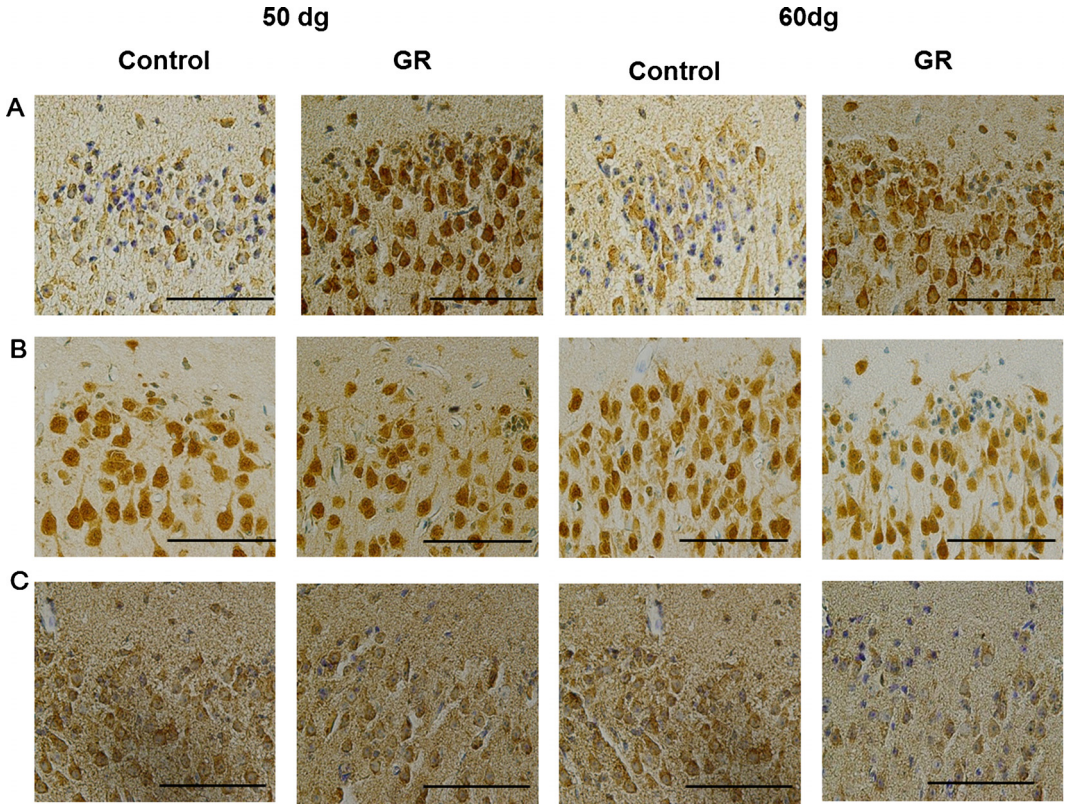

Fig. 1. Representative photomicrographs of HIF1α (A), NeuN (B), and BDNF (C) immunoreactivity in the cerebral parietal cortex from a control and a GR fetus at 50 and 60 dg. HIF1α-IR cells in the GR groups were strongly stained in sections at 50 and 60 dg. NeuN-IR cells in the GR group were smaller and fewer in density than in the control group at 60 dg. The density of BDNF-IR cell was lower in GR fetuses than in controls at 60 dg. Scale bars = 100 μm.

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