Int. J. Devl Neuroscience 53 (2016) 35–45

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Environmental enrichment attenuates the blood brain barrier dysfunction induced by the neonatal hypoxia-ischemia

Ramiro Diaz[a], Patrícia Maidana Miguel[a], Bruna Ferrary Deniz[a], Heloísa Deola Confortim[a], Sílvia Barbosa[a,b], Monique Culturato Padilha Mendonça[c,d], Maria Alice da Cruz-Höfling[c,d], Lenir Orlandi Pereira[a,b,*]

[a] Programa de Pós Graduação em Neurociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Departamento de Ciências Morfológicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil
[d] Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia (IB), Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 8 June 2016
Accepted 16 June 2016
Available online 17 June 2016

Keywords:
Neonatal asphyxia
Enriched environment
Endothelial junctions
Brain
Perinatal brain damage
Neuroprotection

ABSTRACT

Environmental enrichment (EE) is considered an efficient neuroprotector against neonatal hypoxia-ischemia (HI). Nevertheless, the mechanisms involved are not yet clear. In this context, the aim of this study was to investigate the effects of neonatal HI and environmental stimulation in the hippocampus of rats at 3 different time points (PND 8, 22 and 60), evaluating some aspects of BBB structure and function. Seven-day-old Wistar rats were divided into four groups: a control group maintained in a standard environment (CTSE), a control group maintained in an enrichment environment (CTEE), an HI group maintained in a standard environment (HISE) and an HI group maintained in an enrichment environment (HIEE). At the 7th postnatal day (PND), rats were submitted to the Levine-Rice model of neonatal HI. This method consists of permanent occlusion of the right common carotid artery with subsequent exposure to hypoxia. Rats from CTEE and HIEE were stimulated with environmental enrichment. The EE protocol started 24 h after HI, in which pup rats with their dams were stimulated in a maintained EE (PND 8–22). Subsequently, animals were submitted to daily EE (1 h/day, PND 23–60). The expression of some proteins involved in BBB structure (β-catenin, occludin, connexin-43, aquaporin-4, glut-1 and GFAP) were quantified by western blotting in the hippocampi of rats in three periods, at PND 8, 22 and 60. The BBB permeability and integrity was assessed by Evans blue staining and the immunohistochemistry for GFAP in the CA1 region of the hippocampus were also performed. The results showed an HI-induced decreased occludin expression at PND 22 and low levels of occludin, β-catenin and GFAP at PND 60 in the hippocampus of the hypoxic-ischemic rats. Interestingly, in young and adult rats, EE reversed these effects. Evans blue extravasation into the brain parenchyma confirmed the BBB dysfunction brought on by HI. No differences were observed at PND 8, probably due to the immaturity of the BBB at this age. The present study makes an important contribution to understanding the mechanism of the hypoxic-ischemic brain damage and also to presents, for the first time, the recovery of BBB dysfunction as a possible pathway for the protective effect of EE.

Abbreviations: AJs, adherens junctions; ANOVA, analysis of variance; BBB, blood-brain barrier; CTEE, control exposed to environmental enrichment; CTSE, control maintained in standard environment; EE, environmental enrichment; GFAP, glial fibrillary acidic protein; GLUT1, glucose transporter 1; HI, hypoxia-ischemia; HIEE, hypoxia-ischemia exposed to environmental enrichment; HISE, hypoxia-ischemia maintained in standard environment; PND, postnatal day; TJs, tight junctions.

* Corresponding author at: Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500 sala 107, 90050-170, Porto Alegre, RS, Brazil.
E-mail addresses: rdfisioterapia@gmail.com (R. Diaz), patymiguel@msn.com (P.M. Miguel), bruninhaferrary@hotmail.com (B.F. Deniz), heloisadconfortim@gmail.com (H.D. Confortim), silvia.barbosa@ufrgs.br (S. Barbosa), mo_padilha@hotmail.com (M.C.P. Mendonça), hofling@unicamp.br (M.A. da Cruz-Höfling), lenir.orlandi@ufrgs.br (L.O. Pereira).

http://dx.doi.org/10.1016/j.ijdevneu.2016.06.006
0736-5748/

1. Introduction

Neonatal encephalopathy has an incidence rate around 1.5 per 1000 live births. It is estimated that 30% of cases occur in developed countries and 60% of cases occur in developing countries, associated with intrapartum hypoxia-ischemia (HI) (Kurinczuk et al., 2010). The resulting brain damage is frequently associated with cognitive and motor deficits, which are maintained throughout life (Chan et al., 2010, 2015; Johnston et al., 2001; Van de Looij et al., 2015; Yang et al., 2009). The main mechanisms from hypoxia involve cell death and energy depletion, followed by reperfusion, oxidative stress and glutamatergic excitotoxicity. In the sequence, a process of persistent inflammation and epigenetic changes can cause a delay in glial cell maturation, impaired neurogenesis and synaptogenesis with delayed cell death, primarily affecting the hippocampus and other cortical brain structures (Fleiss and Gressens, 2012; Juul and Ferriero 2014).

There is consensus that brain ischemic events disrupt blood-brain barrier (BBB) structure and function (Chen et al., 2015; Li et al., 2015; Won et al., 2011). The existence of this functional barrier in the cerebrovascular system is crucial for regulating the exchange of molecules between the blood and the brain (Boulay et al., 2015). It is also important to preserve the homeostasis of the neural microenvironment necessary for proper neural communication and survival (Abbott et al., 2006). The BBB is constituted by a physical barrier formed by a unique endothelial junctional complex (with adherens junctions and tight junctions) and a functional molecular barrier composed of membrane receptors and transporters and vesicular trafficking mechanisms (Abbott et al., 2010). The tight junctions (TJs) comprise the proteins occludin and claudin and are responsible for controlling paracellular transport, while the adherens junctions (AJs) have the role of supporting TJs by providing structural integrity and attachment between endothelial cells (Abbott et al., 2010). In the formation of AJs, transmembrane cadherin proteins are linked to the cell cytoplasm by catenin proteins (Wolburg and Lippoldt, 2002). Additionally, perivascular astrocytic end feet processes establish strong association with the endothelium of brain capillaries, being such association fundamental for the regulation and maintenance of the BBB functionality. Such association allows the astrocytic perivascular processes to distribute the glucose that is taken by the barrier-type endothelium that lines the brain capillaries from peripheral circulation to be delivered into neuropile. The distribution of glucose by means of the astroglial network is mediated by gap junction channels whose main former protein in astrocytes is connexin 43 (Persidsky et al., 2006; Rouach et al., 2008). Astrocytes also contribute to barrier function under physiological conditions and support the main components of the barrier in cases of breakdown (Wolburg et al., 2009). Astrocytes also contribute to depositing the perivascular extracellular matrix, i.e., the basal lamina (Menezes et al., 2014), which is directly connected to the functional barrier endothelium. Illustrating this connection, it was demonstrated that disruption of the basal lamina results in an altered distribution of aquaporin 4 (AQP4), a water channel present at the adluminal glial end feet membrane that controls the flux of water between the blood and the brain and is responsible for edema formation and resolution in the brain (Nagelhus and Ottersen, 2013). Although it is frequently cited that the BBB is altered after neonatal HI, only a small number of studies have explored this idea. It has been shown that damage to the structure and function of the BBB occurs early, starting 2 h after HI, with extravasation of immunoglobulin G (Lee et al., 2012). Wu et al. (2013) and Muramatsu et al. (1997) also identified extravascular immunoglobulin G in hypoxic-ischemic rats, indicating increased BBB permeability. BBB leakage was also evaluated by albumin extravasation in rats submitted to neonatal HI at PND 12 (Brochu et al., 2011). Additionally, Vannucci et al. (1998) identified increased levels of GLUT-1 as an acute response to neonatal HI. It is important to note that none of these studies had the BBB as the main focus. This is a contradictory finding, given the crucial importance of this barrier for brain function and preservation, particularly under disease conditions.

We have investigated the impact of the environmental enrichment (EE) on the consequences of neonatal HI in rats. It was demonstrated that cognitive dysfunction, including spatial and aversive memory, was recovered in adult rats submitted to EE after HI (Pereira et al., 2007; Rojas et al., 2013, 2015); also, in adolescent rats, a brief period of environmental stimulus prevented object recognition memory impairment (Pereira et al., 2008). These neuroprotective functional effects were associated with dendritic spine density preservation and the recovery of Na^+K^+-ATPase activity (Rojas et al., 2013, 2015). Taken together, these findings give further support for the use of environmental stimulation after neonatal brain damage. However, the mechanisms of this effect are poorly understood.

The present study is founded on the following statements. First, it is notable that hypoxic-ischemic events are clinically relevant and it is crucial investigate therapeutic strategies, their advantages and the mechanisms involved. Second, our previous studies strongly suggest that EE is a good opportunity for the prevention and/or treatment for neonatal brain damage. Finally, there is poor comprehension about the effects of neonatal HI on the BBB during development, and there are no reports on the effects of EE. Therefore, the aim of this study was to investigate the effects of neonatal HI and environmental stimulation in the hippocampus of rats at 3 time points (PND 8, 22 and 60), evaluating some aspects of BBB endothelium structure and function, such as the expression of junctional proteins occludin and β-catenin, and GLUT-1 transporter (also present in astrocytes) and factors related to astrocytes and their functions-expression of glial fibrillary acidic protein (GFAP), 43 (Cx43) and AQP4. Although a few studies have assessed the functionality of the barrier following hypoxic-ischemic injury, we focused on the analysis of alterations to BBB components to understand its function and/or dysfunction. We hypothesized that HI will result in BBB disruption, which can be reversed and/or alleviated by environmental stimulation.

2. Materials and methods

2.1. Animals

Male and female Wistar rats were obtained from the Central Animal House of the Institute of Basic Health Sciences of the Universidade Federal do Rio Grande do Sul. They were maintained in a temperature-controlled room (21± 2 °C), on a 12/12 h light/dark cycle, with food and water available *ad libitum*. On the 7th postnatal day (PND), the animals were randomly assigned to four experimental groups: (1) control, maintained in a standard environment (CTSE); (2) control submitted to EE (CTEE); (3) hypoxia-ischemia and maintained in standard environment (HISE); and (4) hypoxia-ischemia submitted to EE (HIEE). Rats were euthanized at 3 time points (PND 8, 22 and 60) for western blotting and Blue Evans analyses and for immunohistochemistry at PND 22 and 60. For each time point were used 3 female and 3 male rats per group. A pool of two samples was used for western blotting analyses due to small size of hippocampus (atrophy consequent to HI).

All procedures were performed in accordance with the Federation of Brazilian Societies for Experimental Biology and the Guide for the Care and Use of Laboratory Animals adopted by National Institute of Health (USA). The experimental protocol was approved by the Ethics Committee of the Universidade Federal Rio Grande do Sul, Brazil (n.19863).