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Research Report

Neuroprotective effects of hypothermia on synaptic actin cytoskeletal changes induced by perinatal asphyxia

Javier Muñiz[a,1], Juan Romero[a,1], Mariana Holubiec[a,1], George Barreto[b], Janneth González[b], Madeleine Saint-Martin[a], Eduardo Blanco[a,c], Juan Carlos Cavicchia[d], Rocío Castilla[a], Francisco Capani[a,e,*]

[a]Laboratorio de Citoarquitectura y Plasticidad Neuronal, Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" (ININCA), UBA-CONICET, C1122AAJ, Buenos Aires, Argentina
[b]Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, Colombia
[c]Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Facultad de Psicología, Universidad de Málaga, Málaga, Spain
[d]Instituto de Histología y Embriología "Dr. Mario H. Burgos" (IHEM-CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina
[e]Departamento de Biología, Universidad Argentina John F Kennedy, Buenos Aires, Argentina

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ABSTRACT

Cerebral hypoxia–ischemia damages synaptic proteins, resulting in cytoskeletal alterations, protein aggregation and neuronal death. In the previous works, we have shown neuronal and synaptic changes in rat neostriatum subjected to hypoxia that leads to ubi-protein accumulation. Recently, we also showed that, changes in F-actin organization could be related to early alterations induced by hypoxia in the Central Nervous System. However, little is known about effective treatment to diminish the damage. The main aim of this work is to study the effects of birth hypothermia on the actin cytoskeleton of neostriatal post-synaptic densities (PSD) in 60 days olds rats by immunohistochemistry, photooxidation and western blot. We used 2 different protocols of hypothermia: (a) intrahypoxic hypothermia at 15 °C and (b) post-hypoxia hypothermia at 32 °C. Consistent with previous data at 30 days, staining with phalloidin–Alexa^{488} followed by confocal microscopy analysis showed an increase of F-actin fluorescent staining in the neostriatum of hypoxic animals. Correlative photooxidation electron microscopy confirmed these observations showing an increment in the number of mushroom-shaped F-actin staining spines in neostriatal excitatory synapses in rats subjected to hypoxia. In addition, western blot revealed β-actin increase in PSDs in hypoxic animals. The optic relative density measurement showed a significant difference between controls and hypoxic animals. When hypoxia was induced under hypothermic conditions, the changes

*Corresponding author at: Laboratorio de Citoarquitectura y Plasticidad Neuronal, Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" (ININCA), UBA-CONICET, Marcelo T. de Alvear 2270, C1122AAJ, Buenos Aires, Argentina. Fax: +54 11 4508 3888.
[1] These authors contributed equally to this work.
E-mail address: fcapani@fmed.uba.ar (F. Capani).

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observed in actin cytoskeleton were blocked. Post-hypoxic hypothermia showed similar answer but actin cytoskeleton modifications were not totally reverted as we observed at 15 °C. These data suggest that the decrease of the body temperature decreases the actin modifications in dendritic spines preventing the neuronal death.

1. Introduction

Birth hypoxia-ischemia or perinatal asphyxia (PA) is a serious complication with a high mortality and morbidity (McGuire, 2006; Van Bel and Groenendaal, 2008). Following PA, approximately 45% of newborn die and 25% have permanent neurological deficits including cerebral palsy, mental retardation and developmental delay, learning disabilities, visual and hearing problems, and different issues in the school readiness (Hill and Volpe, 1981; Amiel-Tison and Ellison, 1986; Vannucci and Perlman, 1997; Gunn, 2000; Osborne et al., 2004; Shankaran, 2009; Titomanlio et al., 2011)

Dendritic spines are small protrusions emerging from their parent dendrites, and their morphological changes are involved in synaptic plasticity (Fukazawa et al., 2003; Dent et al., 2011; Bae et al., 2012), protein translocation (Ouyang et al., 2005) and may be involved in different brain diseases including hypoxia-ischemia (Gisselsson et al., 2005, 2010; Luebke et al., 2010; Saraceno et al., 2012a). Dendritic spines are characterized for a rich actin cytoskeleton network (Fifkova and Delay, 1982; Capani et al., 2001a, 2008). These small structures are composed of different proteins belonging to several sub-families such as membrane receptors, scaffold proteins, signal transduction proteins and cytoskeletal proteins Shirao and González-Billault (2013). Actin filaments in dendritic spines consist of double helix of actin protomers decorated with Arp 2/3 and ADF/cofilin, and the balance between them is closely related to actin dynamic, which may govern morphological and functional synaptic plasticity (Fukazawa et al., 2003; Pollard and Borisy, 2003; Cingolani et al., 2008; Shirao and González-Billault, 2013). Different kinds of dendritic spines were described based on its shape and their content of actin in adult rat brain. Mushroom-shaped spines have stalks with a clear heads differentiation, stubby spines are thick and have no neck and thin spines are characterized for being long and without neck (Capani et al., 2001c).

For decades, neuroprotective options have been explored, however, at the moment there are currently no effective therapies. Hypothermia has outcome as an important tool to reduce the damage after experimental brain ischemia (Capani et al., 1997, 2001b, 2003; Clark et al., 2009; Sameshima and Ikenoue; 2013). In addition, hypothermia has shown good results although focused only on the therapy for neonatal encephalopathy (Shankaran, 2009; Sameshima and Ikenoue; 2013; Wu and Grotta, 2013).

In previous works, we have observed long term alterations in dendritic spines, high level of ubiquitinization, increment in astrocytes reactivity and alterations in dendritic microtubular organization after PA (Capani et al., 2009; Saraceno et al., 2010; Saraceno et al., 2012b). In addition, recently we have described early modifications in the neostriatum synaptic actin cytoskeleton. In this report we aimed to investigate whether hypothermia can prevent alterations in the actin cytoskeleton of dendritic spines after 60 days of induction of PA using correlative light and electron microscopy for phalloidin–eosin and western blot analysis. We used to different protocols of hypothermia: (a) intrahypoxic hypothermia, since we have obtained in the last years clear evidence of its efficiency to totally block hypoxic damage, and (b) post hypoxia hypothermia at 32 °C.

2. Results

2.1. *Effect of temperature on neostriatal cell survival in vivo*

Staining of neostriatal sections with cresyl violet revealed clear nuclear condensation after 2 months in rats subjected to 20 min of PA (Fig. 1A). Slight nuclear condensation was also observed after 10 and 15 min of PA (Fig. 1A). To determine the nature of the condensed cells, a conventional electron microscopy study was performed. We observed that most cells showing nuclear condensation have morphological characteristics corresponding to neurons in degeneration, such as dark cytoplasm with vacuoles, nucleus compaction, a nucleus with a festoon shape and twisted nuclear envelope (Capani et al., 1997, 2009; Aggoun-Zouaoui et al., 1998; Liu et al., 2004). In contrast, these ultrastructural alterations were not observed in neither using both protocol of hypothermic or control groups (Fig. 2B).

2.2. *Analysis of striatal GABAergic neuronal loss*

To quantify the loss of neurons in neostriatum we employed stereology combined with calbindin immunostaining, which identifies GABAergic neurons in neostriatum (Van den Berg et al., 2003; Capani et al., 2009). We focused only on GABAergic neurons since they represent the target of glutamate synapses from the cortex. Statistics indicated lower means of calbindin IR neurons as compared to the control group, the longer the exposure time of PA was. The overall ANOVA was significant ($P < 0.05$) and post hoc tests showed that the decrement of the means of calbindin IR neurons was statistically significant only at 20 min of PA ($P < 0.05$) while the mean of the HYP 20 min and HYP 20 min 32 °C group were not significantly different from the control group (see Table 1).

2.3. *F-actin staining in neostriatum dendritic spines*

Punctuate staining, representative of areas rich in dendritic spines, was observed at confocal level using phalloidin–Alexa^{488}. Increase in punctuate staining was observed after 20 min of PA,