Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres
Brain Research

Research Report

The neuroprotective actions of hypoxic preconditioning and postconditioning in a neonatal rat model of hypoxic–ischemic brain injury

*Adam A. Galle, Nicole M. Jones**

Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia

ARTICLE INFO

Article history:
Accepted 18 December 2012
Available online 27 December 2012

Keywords:
Hypoxia–ischemia
Neonatal brain injury
Hypoxia
Preconditioning
Postconditioning
Neuroprotection

ABSTRACT

Perinatal hypoxic–ischemic (HI) brain injury remains a major contributing factor to newborn mortality and morbidity. Preconditioning with mild hypoxia has been shown to protect the brain against HI insults and it has recently been shown that mild hypoxia administered after a brain injury, termed 'postconditioning' can protect the adult mouse brain. Here, we have investigated the neuroprotective effects of hypoxic pre- and postconditioning in a neonatal rat model of HI brain injury. 7-Day-old Sprague–Dawley rat pups underwent unilateral common carotid artery ligation in combination with 3 h at 5.5% oxygen. Hypoxic treatments consisted of either 3 h of 8% oxygen performed 24 h prior to injury (preconditioning); or 1 h of 8% oxygen 24 h post-injury, performed once a day for 5 days (postconditioning). Brains were removed 1 week post-injury for histological analysis. HI caused an increase in lesion volume compared to controls and both hypoxic pre- and postconditioning reduced the degree of brain damage following HI injury. To specifically examine neuronal loss, NeuN immunohistochemistry and regional brain area analysis were performed. HI injury caused a loss in NeuN staining in all brain regions examined. Preconditioning with hypoxia resulted in a significant reduction in cortical, hippocampal and striatal neuronal loss, compared with HI alone. Hypoxic postconditioning resulted in a reduction in cortical and striatal neuronal loss, compared to HI alone. Our results further support the clinical potential for mild hypoxia in the treatment of brain injuries, either as a pre- or post-injury treatment strategy.

1. Introduction

Perinatal hypoxic–ischemic (HI) brain injury is a leading cause of acute mortality and chronic disability in newborns, occurring in approximately 1–2 in 1000 live births (Grafe et al., 2008). Of the total number of infants who survive a HI insult, up to 50% die during the postnatal period, and of those infants who survive, up to 25% can go on to develop an

Abbreviations: AM, adrenomedullin; ANOVA, analysis of variance; DAB, 3,3′-diaminobenzidine; HI, hypoxia–ischemia; HIF-1, hypoxia inducible factor 1; HIF-1α, hypoxia inducible factor 1-α; HRP, horseradish peroxidase; IP, intraperitoneal; NeuN, neuronal specific nuclear protein; TBS, tris buffered saline

*Corresponding author. Fax: +61 2 9385 1059.
E-mail address: n.jones@unsw.edu.au (N.M. Jones).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.12.026

ongoing neurological condition or suffer cognitive impairment (Rennie et al., 2007; Tagin et al., 2012). Whilst research over the last two decades has done much to unravel the pathologic sequelae of HI brain injury, currently, there are relatively few options available for clinical treatment. The only intervention yet to have shown potential in humans following perinatal HI brain injury is induced whole-body or head-selective hypothermia (Jacobs et al., 2007; Laptook, 2010). Hypothermia appears to have beneficial effects in those infants who are moderately–severely affected by hypoxic–ischemic encephalopathy (Edwards et al., 2010; Wachtel and Hendricks-Munoz, 2011; Tagin et al., 2012). At present, the mechanisms involved in hypothermia induced protection are still being explored and the longer term outcomes are not yet known, therefore it is still important to examine other possible therapeutic strategies.

One potential neuroprotective strategy in animal models of perinatal HI injury is hypoxic preconditioning (Gidday et al., 1994). The main premise of preconditioning centers on the concept that exposure to a mild form of physiological stress; in this case a non-injurious level of hypoxia can protect the brain against a subsequent insult (Gidday et al., 1994; Jones et al., 2008). Hypoxic preconditioning has been extensively investigated over the last 17 years and whilst neuroprotection is a clear outcome of this treatment, it has been in more recent studies that have indicated that hypoxia inducible gene activation might contribute to this protection (Bernaudin et al., 2002; Jones and Bergeron, 2001). Previous studies have demonstrated the ability of tissue hypoxia to induce the expression of genes involved in a range of adaptive and ultimately protective processes, including erythropoiesis, angiogenesis, glucose metabolism and cell proliferation

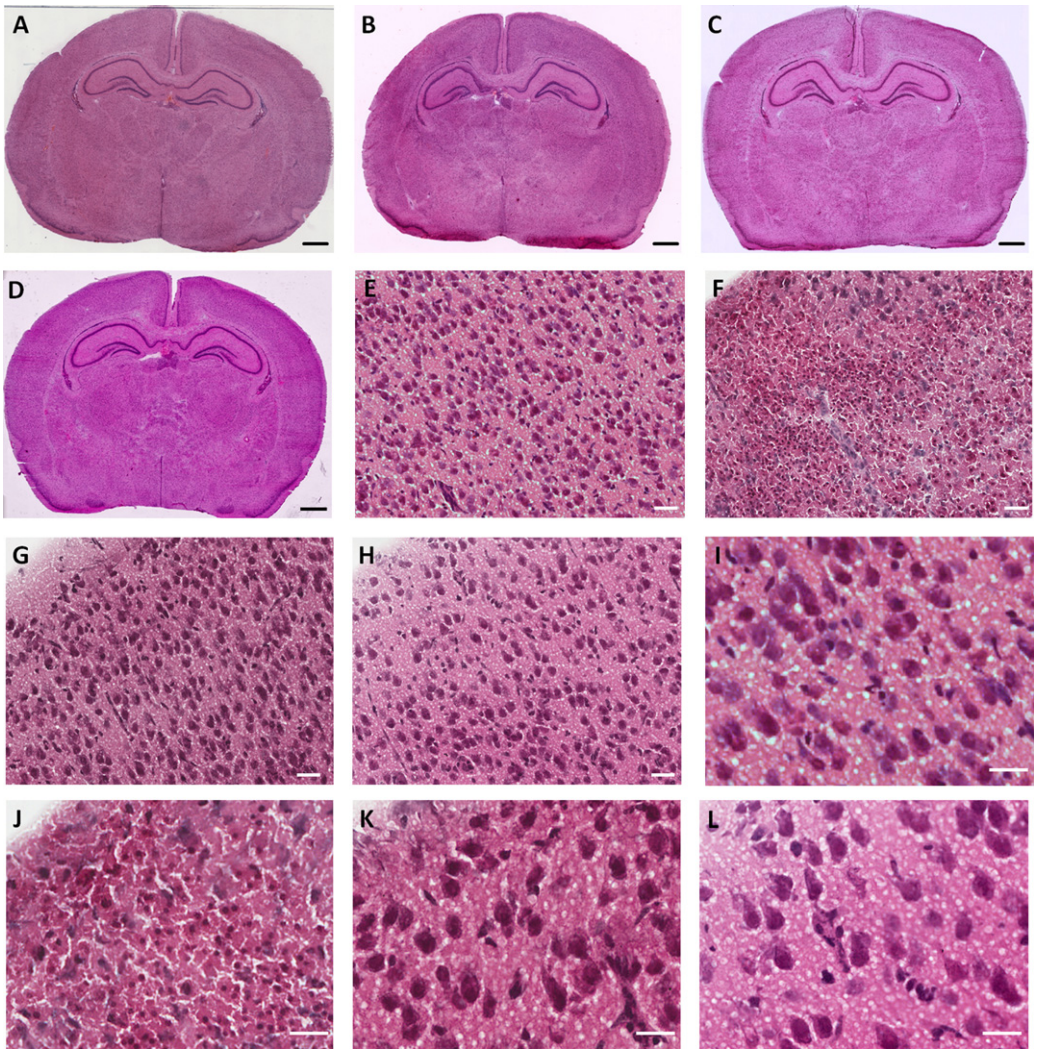

Fig. 1 – Photomicrographs demonstrating the neuroprotective effects of hypoxic preconditioning and postconditioning, following HI insult. Brains were collected 1 week after HI and coronal brain sections were stained with hematoxylin and eosin to illustrate the damage to the whole brain (A–D), cortex ipsilateral to the HI injury (E–H) and cortex ipsilateral to HI injury at higher magnification (I–L). HI resulted in shrinkage of the ipsilateral (left) hemisphere (B), which was not seen in control animals (A), and markedly reduced in the preconditioned (C) and postconditioned (D) brains. Preconditioning and postconditioning with hypoxia also reduced the amount of neuronal loss, disruption of the cortical layers and infiltration of non-neuronal cells following HI (G and H respectively), similar to uninjured animals (E), when compared with controls (F). Scale bar = 1 mm (A–D), 50 μm (E–H) and 25 μm (I–L).