Experimental Neurology 286 (2016) 93–106

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Neuronal prolyl-4-hydroxylase 2 deficiency improves cognitive abilities in a murine model of cerebral hypoperfusion

Daniel Gruneberg [a], Felipe A. Montellano [a], Konstanze Plaschke [b], Lexiao Li [a], Hugo H. Marti [a], Reiner Kunze [a,*]

[a] Institute of Physiology and Pathophysiology, University of Heidelberg, Germany
[b] Department of Anesthesiology, University of Heidelberg, Germany

ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form 14 September 2016
Accepted 4 October 2016
Available online 05 October 2016

Keywords:
HIF
PHD
Epo
VEGF
Hippocampus
Learning
Memory
Alzheimer's disease

ABSTRACT

Episodes of cerebral hypoxia/ischemia increase the risk of dementia, which is associated with impaired learning and memory. Previous studies in rodent models of dementia indicated a favorable effect of the hypoxia-inducible factor (HIF) targets VEGF (vascular endothelial growth factor) and erythropoietin (Epo). In the present study we thus investigated whether activation of the entire adaptive HIF pathway in neurons by cell-specific deletion of the HIF suppressor prolyl-4-hydroxylase 2 (PHD2) improves cognitive abilities in young (3 months) and old (18–28 months) mice suffering from chronic brain hypoperfusion. Mice underwent permanent occlusion of the left common carotid artery, and cognitive function was assessed using the Morris water navigation task. Under conditions of both normal and decreased brain perfusion, neuronal PHD2 deficiency resulted in improved and faster spatial learning in young mice, which was preserved to some extent also in old animals. The loss of PHD2 in neurons resulted in enhanced hippocampal mRNA and protein levels of Epo and VEGF, but did not alter local microvascular density, dendritic spine morphology, or expression of synaptic plasticity-related genes in the hippocampus. Instead, better cognitive function in PHD2 deficient animals was accompanied by an increased number of neuronal precursor cells along the subgranular zone of the dentate gyrus. Overall, our current pre-clinical findings indicate an important role for the endogenous oxygen sensing machinery, encompassing PHDs, HIFs and HIF target genes, for proper cognitive function. Thus, pharmacological compounds affecting the PHD-HIF axis might well be suited to treat cognitive dysfunction and neurodegenerative processes.

1. Introduction

In response to declining oxygen levels, cells rapidly activate hypoxia-inducible transcription factors (HIFs). HIFs are heterodimers consisting of an α and β subunit, and specifically bind to hypoxia-response elements (HRE) in the promoters and enhancers of numerous genes involved in glycolysis, angiogenesis, and cell survival promoting adaptation to hypoxic-ischemic stress (Rabie and Marti, 2008). The family of prolyl-4-hydroxylase domain (PHD) proteins and factor inhibiting HIF (FIH), whose activity is dependent on the availability of molecular oxygen, ferrous iron and 2-oxoglutarate, are the primary regulators of HIF function. Under normoxic conditions, HIF-α subunits are hydroxylated on conserved proline and asparagine residues by PHDs and FIH, respectively. While prolyl hydroxylation causes recruitment of the von Hippel-Lindau protein E3 ubiquitin ligase and immediate proteasomal degradation of HIF-1α and -2α, asparaginyl hydroxylation prevents interaction with the co-activators p300 and CREB-binding protein (CBP), reducing its transcriptional activity (Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001; Kaelin and Ratcliffe, 2008; Lando et al., 2002a; Lando et al., 2002b). In contrast, PHDs and FIH are less active during hypoxic conditions resulting in accumulation and nuclear translocation of hypohydroxylated HIF-α. In nuclei the functional HIF complex is formed by dimerization with constitutively expressed HIF-1β followed by HRE binding and recruitment of p300/CBP resulting in enhanced transcription of HIF-responsive genes (Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001; Kaelin and Ratcliffe, 2008; Lando et al., 2002a; Lando et al., 2002b).

Tissue hypoxia is the shared feature in a variety of human diseases affecting the brain, such as stroke (ischemic hypoxia) (Dirnagl et al., 1999), Alzheimer's disease (AD) and vascular dementia (VaD) (hypoperfusion) (Gao et al., 2013; Raz et al., 2016), or even sleep apnea (arterial hypoxia) (Daulatzai, 2013). Depending on the severity, cerebral hypoxia will lead to neuronal dysfunction, cognitive impairment and finally cell death. A growing number of studies demonstrate that episodes of cerebral hypoxia/ischemia increase the risk to develop dementia (Zhang and Le, 2010). On the other hand, brain hypoxia will rapidly induce the HIF system and initiate a defending response. The HIF-targets

Abbreviations: CCAO, cerebral carotid artery occlusion; Epo, erythropoietin; HIF, hypoxia-inducible factor; PHD, prolyl-4-hydroxylase domain; VEGF, vascular endothelial growth factor.

* Corresponding author at: University of Heidelberg, Institute of Physiology and Pathophysiology, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany.
E-mail address: reiner.kunze@physiologie.uni-heidelberg.de (R. Kunze).

http://dx.doi.org/10.1016/j.expneurol.2016.10.001
0014-4886/

vascular endothelial growth factor (VEGF) and erythropoietin (Epo) are up-regulated in neurons and other cells of the central nervous system (CNS) under hypoxic-ischemic conditions, and exhibit potent cytoprotective, angiogenic, neurogenic and neurotrophic properties (Greenberg and Jin, 2013; Rabie and Marti, 2008; Sun et al., 2003). In diverse animal models of AD and VaD, we and others have shown that gain of function of VEGF can attenuate memory and learning impairment (Herran et al., 2013; Plaschke et al., 2008; Religa et al., 2013; Spuch et al., 2010; Wang et al., 2011). Similar findings have been reported for Epo (Al-Qahtani et al., 2014; Armand-Ugon et al., 2015; Esmaeili Tazangi et al., 2015; Hamidi et al., 2013; Lee et al., 2012; Maurice et al., 2013).

We hypothesize that targeted activation of the HIF system by selective ablation of *Phd2* in neurons would counteract cognitive impairment induced by brain hypoperfusion. To address this, an established mouse oligemia model (Plaschke et al., 2008) was used, and cognitive testing was performed in young and aged mice with neuron-specific ablation of *Phd2* (Kunze et al., 2012).

2. Material and methods

2.1. Animal experiments

All animal experiments were approved by the local animal welfare committee (Regierungspräsidium Karlsruhe, Germany, approval number: 35-9185.81/G-103/12), conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and were performed in accordance with the recently published Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines (http://www.nc3rs.org/ARRIVE). All mice used in the animal experiments were randomized. The operator or investigator was blinded for the respective mouse genotypes throughout the study. Evaluation of all read-out parameters were done independently and in a blinded fashion.

2.2. Generation of neuron-specific Phd2 and Phd2/Hif1a/Hif2a knockout mice

All mice were maintained at the animal facility of the University of Heidelberg under specific pathogen-free conditions, a controlled and inverted 12:12 hour light-dark cycle (lights on at 7 PM and off at 7 AM), constant room temperature (22 ± 2 °C) and relative humidity (50–55%) with food and water ad libitum. *Phd2* $^{f/f}$ mice were bred with a transgenic mouse line expressing Cre recombinase under control of the Ca^{2+}/calmodulin-dependent protein kinase IIα promotor (*CaMKIIα:cre*) to generate mice with a *Phd2* knockout restricted to postnatal forebrain neurons (*nPhd2* $^{\Delta/\Delta}$) (Casanova et al., 2001; Kunze et al., 2012). *Hif1a* $^{f/f}$ (Ryan et al., 2000) and *Hif2a* $^{f/f}$ mice (Gruber et al., 2007) were initially intercrossed and resulting *Hif1a/Hif2a* $^{ff/ff}$ mice were mated with *nPhd2* $^{\Delta/\Delta}$ mice to obtain homozygous forebrain neuron-restricted knockout mice (*nPhd2/Hif1a/Hif2a* $^{\Delta\Delta\Delta/\Delta\Delta\Delta}$). Animals were genotyped using primers (Eurofins Genomics, Ebersberg, Germany) described in Table S1.

2.3. Unilateral common carotid artery occlusion (CCAO)

Male mice (young: 10–17 week old, aged: 73–113 week old) were anesthetized by a mixture containing 2% isoflurane, 70% N_2O, and remainder O_2, and were maintained by reducing the isoflurane concentration to 1.0–1.5%. Core body temperature was maintained at 37 °C throughout surgery by using a feedback-controlled heating device. The surgery was performed as described previously (Plaschke et al., 2008). Briefly, the left common carotid artery was exposed by a ventral medial incision and was gently isolated from the carotid sheath and vagus nerve. The artery was ligated just below bifurcation twice with 8–0 silk sutures (Johnson & Johnson Medical GmbH, Norderstedt, Germany) and cut in between. Finally, the midline incision was carefully closed. For sham surgery the mice underwent the same procedure without vessel occlusion. Following operation the animals were transferred back into their cages with free access to water and food. Some hours before and after surgery animals were maintained under normal day-night cycle (lights on at 7 AM and off at 7 PM). Table S2 lists the criteria resulting in exclusion from analyses.

2.4. Modified holeboard testing

A modified holeboard test was used to test for differences in spontaneous exploration behavior and locomotor function before the animals were subjected to the Morris Water Maze test (Plaschke et al., 2008). Mice were placed in a black box (70 × 70 × 40 cm) containing 16 holes in a 4 × 4 array on a flat surface. Holes provided infrared photoelectric barriers to detect nose-poke events at every hole over a total duration of 300 s. Latency to the first contact, total number of contacted holes and moved distance were calculated by AKS registration system (TSE Systems, Bad Homburg, Germany). The experimental set-up was cleaned after every trial to avoid olfactory disturbances in following animals.

2.5. Morris Water Maze testing

The Morris Water Maze navigation task was performed as described previously with some minor changes (Plaschke et al., 2008). Young and aged animals were tested independently from each other by two different investigators, who were blinded for the respective mouse genotypes throughout the complete study.

2.5.1. Morris Water Maze apparatus

A white-colored round basin (Noldus Information Technology, Freiburg, Germany, 150 cm in diameter, 70 cm in depth) was filled with water (22 ± 1 °C) up to 10 cm below the rim. The tank was virtually divided into four equal quadrants. A clear-transparent platform of acrylic glass (10 cm × 10 cm) was submerged to 3 mm below the water surface and placed in the middle of the target quadrant. Visual cues (15 cm × 15 cm, black cue on white background) were placed around the basin in following positions: north, west and east. A closed-circuit digital camera (Philips AG, Hamburg, Germany) was mounted on the ceiling above the center of the pool for live recording of the animal swimming trajectories. Data was evaluated using EthoVision 8.5 video tracking software (Noldus Information Technology).

2.5.2. Morris Water Maze procedure

The animals were first allowed to readapt to a reversed day-night cycle (lights on at 7 PM and off at 7 AM) for 3 days. Then, mice were tested during the night phases of five consecutive days in a dark room equipped with background lightning. Each animal had three to five training trials per day separated by a 30–40 min inter-trial interval. Mice were placed into water at defined locations to find the platform. Data capturing started automatically through an optical trigger, and the experimenter left the room to avoid any disturbance. Finding the platform was defined as staying on it for at least 4 s before the time frame of 75 s ended (time to platform, ttp). If the mouse failed to find the platform in the defined time window, it was placed onto the platform for 10 s, and assigned a latency of 75 s. Subsequently, mice were allowed to dry and warm up under an infrared lamp.

2.5.3. Visible platform (Cued trial)

The cued trial was applied to assess the mice's motivation to escape from the water as well as their sensorimotor integrity. The clear-transparent platform was placed in the north quadrant and was marked by a visible cue (yellow striped cylinder at the top, 13 cm high, 3 cm in diameter). Each animal was tested in 4 single trials during the first training day.