Biochimica et Biophysica Acta 1834 (2013) 1772–1778

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Neuroglobin involvement in visual pathways through the optic nerve ☆

Christophe Lechauve *, Sébastien Augustin [1], Delphine Roussel [1], José-Alain Sahel, Marisol Corral-Debrinski

INSERM, U968, Paris F-75012, France
UPMC Univ Paris 06, UMR-S 968, Institut De la Vision, Paris F-75012, France
CNRS, UMR-7210, Paris F-75012, France
Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, INSERM-DHOS CIC 503, Paris F-75012, France

ARTICLE INFO

Article history:
Received 3 December 2012
Received in revised form 26 February 2013
Accepted 15 April 2013
Available online 29 April 2013

Keywords:
Neuroglobin
Mitochondria
Retinal ganglion cell
Nerve axon
Optic nerve
Glial cell

ABSTRACT

Neuroglobin is a member of the globin superfamily proposed to be only expressed in neurons and involved in neuronal protection from hypoxia or oxidative stress. A significant fraction of the protein localizes within the mitochondria and is directly associated with mitochondrial metabolism and integrity. The retina is the site of the highest concentration for neuroglobin and has been reported to be up to 100-fold higher than in the brain. Since neuroglobin was especially abundant in retinal ganglion cell layer, we investigated its abundance in optic nerves. Remarkably in optic nerves, neuroglobin is observed, as expected, in retinal ganglion cell axon profiles but also astrocyte processes, in physiological conditions, possess high levels of the protein. Neuroglobin mRNA and protein levels are ~10-fold higher in optic nerves than in retinas, indicating an important accumulation of neuroglobin in these support cells. Additionally, neuroglobin levels increase in Müller cells during reactive gliosis in response to eye injury. This suggests the pivotal role of neuroglobin in retinal glia involved in neuronal support and/or healing. This article is part of a Special Issue entitled: Oxygen Binding and Sensing Proteins.

1. Introduction

Neuroglobin (Ngb) is a globin expressed in neurons of the central and peripheral nervous systems [1]. Ngb is proposed to be a neuron-specific protein but seems also to be expressed in reactive and scar-forming astrocytes during a pathological process [2]. Constitutive Ngb expression in the mammalian central nervous system (CNS) is observed in neurons from the cerebellum, brainstem, hypothalamus, hippocampus, and cerebral cortex. Furthermore, Ngb was detected in the anterior and posterior eye segments including corneal epithelium, pigmented epithelial cells, photoreceptor inner segments, plexiform layers, and ganglion cell layer (GCL) [1,3,4]. Ngb could be involved in oxidative stress alleviation, reactive oxidative species elimination [5,6] and the preservation of mitochondrial function by preventing apoptosis [7,8].

We recently established that a large amount of Ngb localizes to the mitochondrial matrix or the matrix side of the mitochondrial inner membrane. In addition, the protein is required for the integrity of respiratory chain complexes I and III enzymatic activities [9]. Since retinal ganglion cells (RGCs) accumulate high levels of *NGB* mRNA and protein, we decided to evaluate *NGB* expression in optic nerve. Optic nerve is a typical CNS white matter tract which contains RGC axons (optic fibers) and glial cells namely oligodendrocytes, astrocytes, and microglia. Optic nerve is also the entry point of blood vessels that open into the retina to vascularize the retinal neuron layers. We demonstrated that in adult rats Ngb is present in optic nerve RGC axons and glial cells. Moreover, *NGB* mRNA and protein levels are ~10-fold higher in optic nerves than in retinas, suggesting an important accumulation of Ngb in optic fiber support cells. Moreover, eye lens injury was accompanied by Müller glial cell activation in retinas and we observed the increase of *NGB* expression in these activated cells. Hence, our data show that: (i) Ngb accumulates at high levels in optic nerve glial cells in physiological conditions and; (ii) Ngb levels increase in Müller cells during reactive gliosis (reactive changes of glial cells in response to damage) due to eye lens injury.

2. Materials and methods

2.1. Animals

Male Long Evans rats were used (Janvier, Le Genest St. Isle, France). They were housed in a temperature and humidity controlled environment, two per cage, with a 12 h light–dark cycle, and permanent and unrestricted access to food and water. Few animals developed lens opacification and traumatic injury due to the surgery which can be confirmed by the impossibility to obtain eye fundus imaging by confocal scanning laser ophthalmoscopy [9,10]. All animal studies were conducted in accordance with the guidelines issued by the French Ministry of Agriculture and the Veterinarian Department of Paris

☆ This article is part of a Special Issue entitled: Oxygen Binding and Sensing Proteins.
* Corresponding author at: 17 rue Moreau, 75012 Paris, France. Tel.: +33 1 53 46 25 59; fax: +33 1 53 46 25 02.
E-mail address: christophe.lechauve@inserm.fr (C. Lechauve).
[1] These authors contributed equally to this work.

1570-9639/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbapap.2013.04.014

(Permit number DF/DF_2010_PA1000298), the French Ministry of Research (Approval number 5575) and the ethics committees of the University Paris 6 and INSERM (Authorization number 75-1710).

2.2. Retinal and optic nerve histology

Retinas or optic nerves were fixed in 4% PFA at 4 °C, and cryoprotected by overnight incubation in PBS containing 30% sucrose at 4 °C. Retinas were embedded in optimal cutting temperature compound (Neg 50, Richard-Allan Scientific) and frozen in liquid nitrogen. Optic nerves were incubated in a 7.5% gelatin solution from porcine skin; type A (Sigma-Aldrich) and 10% sucrose and frozen in chilled 2-methyl-butane solution. Retina and optic nerve cryosections with a thickness of 10 μm were obtained using a cryostat (Microm Microtech) and mounted on SuperFrost Plus slides.

For immunochemistry, sections were rinsed with PBS and treated with 1% BSA, 0.1% Triton and 0.05% Tween 20 in PBS for 1 h. They were then incubated with primary antibody overnight at 4 °C. Sections were washed in PBS three times for 5 min and incubated with the appropriate secondary antibody and DAPI (2 μg/mL) for 2 h at room temperature. After three washes with PBS (5 min), cryosections were briefly rinsed with water, and cover slides were mounted using fluoromount solution (Thermo-Scientific). Primary and secondary antibodies used are shown in Table S1.

2.3. Microscopic observations

Fluorescence labeling was monitored with a confocal laser scanning microscope (Olympus FV1000). Microscope control and image acquisition were conducted by using Olympus FluoView® (software

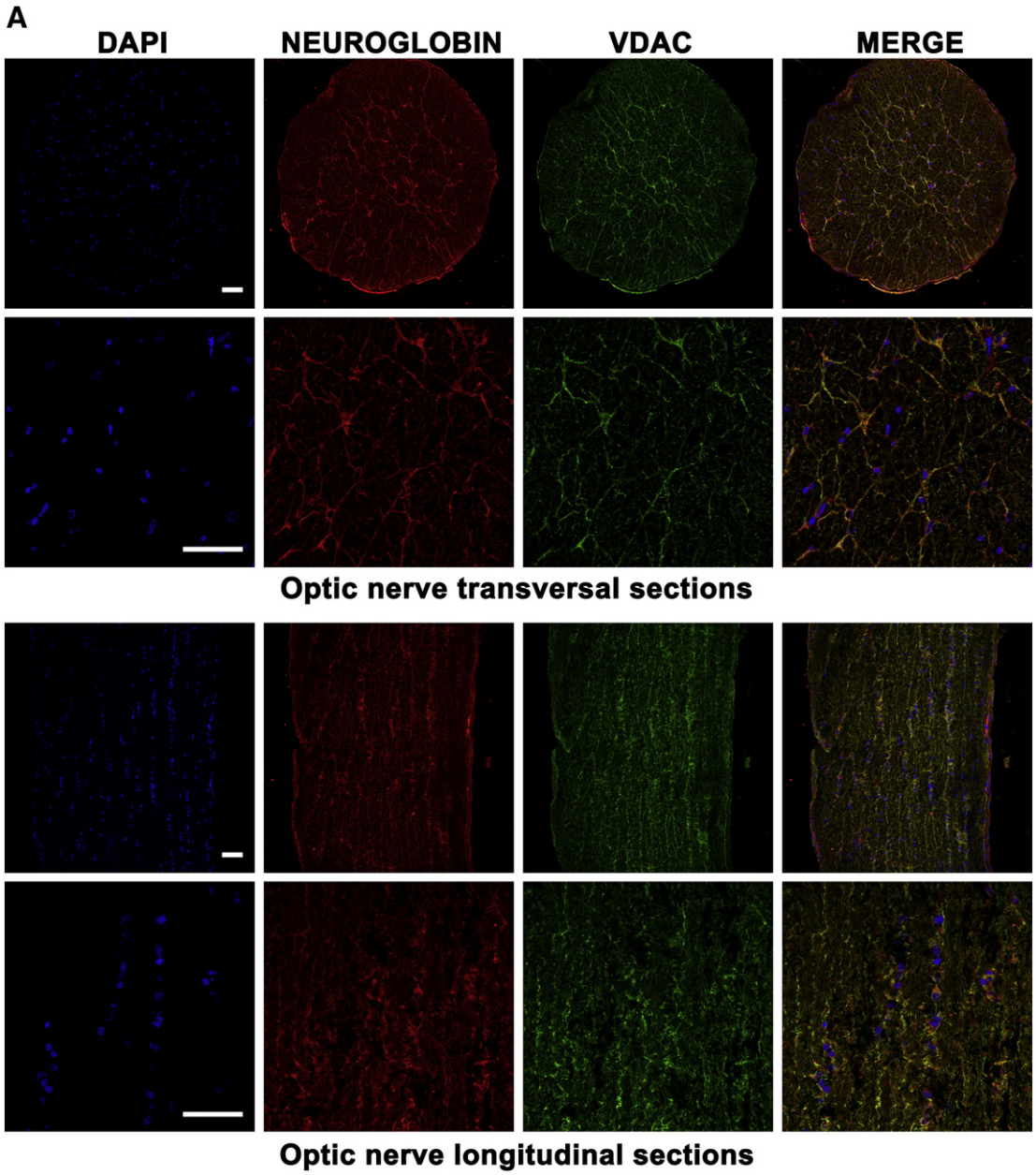

Fig. 1. Immunostaining of neuroglobin in rat optic nerves. (A) The cellular and subcellular localization of Ngb and a mitochondrial protein were examined by indirect immunofluorescence in transversal (upper panel) and longitudinal (lower panel) optic nerve sections using specific antibodies against Ngb (Abcam-ab37258, red) and VDAC (Abcam-ab15895, green) (Table S1). (B) The presence of Ngb in glial cells was examined by indirect immunofluorescence in transversal optic nerve cross sections using antibodies against vimentin (BD Pharmingen-550513, red) or GFAP (Sigma-G3893, red) and Ngb (Sigma, N-7162, green), lower panel. Scale bars represent 50 μm; images were obtained with confocal laser scanning microscope Olympus FV1000.