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Review

Neuroglobin and neuronal cell survival ☆

Marco Fiocchetti [a], Elisabetta De Marinis [a], Paolo Ascenzi [b,c], Maria Marino [a,b,*]

[a] Department of Science, University Roma Tre, Viale Guglielmo Marconi 446, I-00146 Roma, Italy
[b] National Institute of Biostructures and Biosystems, Viale Medaglie d'Oro 305, I-00136 Roma, Italy
[c] Interdepartmental Laboratory of Electron Microscopy, University Roma Tre, Via della Vasca Navale 79, I-00146 Roma, Italy

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ABSTRACT

The balance between neuronal apoptosis and survival sculpts the developing brain and has an important role in neurodegenerative diseases. Thus, the individuation of signals that could modulate the cell death machinery as well as enhance survival in neurons promises to provide multiple points of therapeutic intervention in neurodegenerative diseases. Neuroglobin (NGB), the first nerve globin identified in neuronal tissues of humans, seems to possess a protective role in the brain only after up-regulation. Here, the NGB physiological role in the control of neuronal survival is reviewed. *In vitro* studies suggested that cytosolic NGB could react very rapidly with cytochrome *c* released from mitochondria, thus interfering with the intrinsic pathway of apoptosis. Although very suggestive, these data do not explain either the role of NGB up-regulation in neuroprotection or the recently reported NGB localization into mitochondria. Recently, we identified the steroid hormone 17β-estradiol (E2) as an endogenous modulator of NGB levels in neuroblastoma SK-N-BE cell line. Upon E2 stimulation, NGB reallocates mainly into mitochondria where the association with the mitochondrial cytochrome *c* occurs. Remarkably, E2 treatment before an apoptotic stimulus strongly enhances the NGB:cytochrome *c* association reducing cytochrome *c* release into the cytosol. As a consequence, a decrease of caspase-3 activation and, in turn, of the apoptotic cascade activation take place. Besides E2, other compounds have been reported to up-regulate the NGB expression highlighting the possibility to develop NGB-mediated therapeutic strategies against stroke damage and neurodegenerative diseases. This article is part of a Special Issue entitled: Oxygen Binding and Sensing Proteins.

1. Introduction

In multicellular organisms, cell death and cell survival/proliferation are balanced. Animal development is characterized by rapid proliferation of embryonic cells which then differentiate to specialized cells of adult tissues and organs. This complex process of development involves not only cell proliferation and differentiation but also cell death. In adult organism, cell death must be balanced by cell renewal and most tissues contain stem cells that are able to replace those that have been lost [1,2].

Mammalian neurons are the most long-lived cell types in the body. In the early phases of the nervous system development, a great number of neurons is generated. After a selective period regulated by massive cell death during development, the same neuronal population is constantly maintained during life. Thus, neuronal cell death is the cardinal feature of brain development [1,3]. On the other hand, neuronal survival requires the support of trophic factors which derive from neighboring cells; only those neurons that succeed in establishing correct synaptic connections would obtain stimuli to allow their preservation. According to this idea, neuronal survival requires positive signals suggesting that death is the default fate of neurons [4].

Apoptosis continues throughout life and represents the central mechanism to remove surplus, damaged, and aged cells. Deregulation of the normal apoptotic pathway may play an important role in cancers and various pathologies of the central nervous system. A variety of human neuronal disorders, like as Alzheimer's and Huntington's diseases and amyotrophic lateral sclerosis, are characterized by the gradual loss of a specific subset of neurons via several mechanisms including the lack of specific neurothrophic hormones, calcium influx, oxidative stress and excitotoxicity or a combination of these [5–7]. Considering the central role of neuronal apoptosis in diseases of the central nervous system, several research groups are now focused on the prospect of

Abbreviations: Aβ, amyloid-β peptides; APAF-1, apoptotic protease-activating factor-1; E2, 17β-estradiol; ER, estrogen receptor; Erβ, estrogen receptor β; ERE, estrogen responsive elements; HIF-1, hypoxia-inducible factor-1; mPTP, mitochondria permeability transition pore; NGB, neuroglobin; NGB-Tg mice, NGB-over-expressing transgenic mice; TAT protein, trans-activator of the transcription protein; TAT-NGB, trans-activator of the transcription and NGB; VDAC, voltage-dependent anion channel; VEGF, vascular endothelial growth factor

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* Corresponding author at: Department of Science, University Roma Tre, Viale Guglielmo Marconi 446, I-00146 Roma, Italy. Tel.: +39 06 57336345; fax: +39 06 57336321.
E-mail address: maria.marino@uniroma3.it (M. Marino).

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developing effective therapeutic strategies based on the modulation of this physiological process.

Neuroglobin (NGB), a globin family member, is a monomeric hexa-coordinated heme protein of 17 kDa predominantly expressed in neurons of the central and peripheral nervous system but also recognized in non-neuronal tissues [8–10]. During the past decade, the neuroprotective roles of NGB in a wide range of neurological disorders have been demonstrated. In particular, high NGB levels inversely correlate with the severity of functional deficits linked to neurological diseases as well as with neuron death [8,11–16]. Despite these data, the underlying molecular mechanism(s) remains undefined. NGB-induced plasma membrane-starting signals, NGB:protein interactions into the cytosol, and, more recently, NGB interaction with mitochondrial proteins have all been evoked as the bases of NGB's neuroprotection.

Here, molecular mechanisms involved in NGB's neuroprotection with particular attention to mitochondrial pathways committed to apoptosis and cell survival are reviewed taking into account both *in vitro* and *in vivo* studies.

2. NGB levels and neuroprotection

The brain is usually exposed to a wide variety of stimuli and can activate several endogenous protection pathways in which a large number of proteins, including hypoxia-inducible factor-1 (HIF-1), pyruvate dehydrogenase kinase 1, and NGB, are directly involved [17]. Strategies which up-regulate the expression of these macromolecules are expected to be neuroprotective. Remarkably, NGB is a neuroprotective molecule which over-expression could protect cultured neurons and the animal nervous system against several insults [12,15,18–20].

In this respect, the antisense-mediated knock-down of NGB rendered cortical neurons more vulnerable to hypoxia and to oxidative stress, whereas the over-expression of NGB conferred protection to cultured neurons against hypoxia eliminating hypoxia-induced mitochondrial aggregation and neuron death [15,21–24]. Similar effects were observed in human neuroblastoma cell lines SH-SY5Y in which the NGB over-expression enhanced cell survival under conditions of either anoxia or glucose deprivation [14,25].

In primary cultured mouse cortical neurons, NGB over-expression reduces the generation of superoxide anion after a hypoxic insult significantly improving glutathione levels compared with wild type controls [26]. Besides O_2 binding, NGB can also bind and scavenge NO protecting neurons against NO-induced neurotoxicity [21,27]. Moreover, NGB over-expression is associated with reduced reactive oxygen and nitrogen specie production, reduced neuronal injury and lipid peroxidation in a mouse model of ischemia–reperfusion injury [28].

In NGB-over-expressing transgenic (NGB-Tg) mice, the cerebral infarct size after middle cerebral artery occlusion (MCAO) is reduced by 30% compared with wild type controls [13]. Reduction of brain infarction in NGB-Tg mice can be sustained up to 14 days after ischemia compared with wild type controls [29] suggesting that NGB over-expression is neuroprotective against transient focal cerebral ischemia [19]. Recently, NGB was delivered to the mouse brain by using the 11-amino-acid human immunodeficiency virus trans-activator of the transcription protein transduction domain (TAT-NGB). In a mild MCAO mouse model, pre-treatment with TAT-NGB protected brain tissue from ischemic stroke, but no beneficial outcome was observed if the TAT-NGB was administered 2 h after ischemia onset, suggesting that the NGB-over-expression might be beneficial for early stroke treatment and for stroke prevention [20,30]. However, some questions have been raised concerning the capacity of NGB to provide general protection to neurons *in vivo* [31]. In fact, the infarct volume in NGB-null mice was significantly smaller than in wild type controls 24 h after permanent middle cerebral artery occlusion, suggesting that NGB is not protective against ischemic injury, at least at the endogenous expression level [32].

NGB over-expression is also protective against other models of neurological disorders. In fact, by using double transgenic mice expressing a mutant form of human amyloid precursor protein associated with familiar Alzheimer's disease and over-expressing NGB, it has been shown that NGB over-expression inhibits Alzheimer's disease-related raft aggregation, membrane polarization, and associated neuronal death. Moreover, these mice produce reduced amounts of amyloid-β peptides Aβ(1–40) and Aβ(1–42), and amyloid plaques in the brain [33].

As a whole these data point to NGB as one of the endogenous neuroprotective proteins which high levels could protect neurons against death induced by oxidative stress, mitochondrial pathologies, and neurotoxicity.

3. Physiological and pharmacological induction of neuroglobin expression

NGB is principally found in highly metabolically active cells and certain specialized cells, such as neurons of the hypothalamus and in retinal cells [16,34–36]. Under physiological conditions all or at least most neurons of the central nervous system express a low NGB concentration (<1 μM in the mouse brain) [8,9] with the exception of neuronal retina cells in which NGB levels is about 50- to 100-fold higher [8]. Under normal conditions, NGB is barely expressed in resting astrocytes. However, in murine models of traumatic brain injury, cerebral malaria, and experimental autoimmune encephalitis, the NGB expression increases in reactive astrocytes found within brain regions near the pathologies [37]. More recently, NGB immunoreactivity in reactive astrocytes located in proximity of a penetrating cortical injury has been confirmed in mouse primary cortical astrocytes [38]. In cell culture systems, NGB expression is moderately induced by hypoxia (27), H_2O_2 toxicity (49), and lipopolysaccharide [38]. These data suggest that NGB is a stress-inducible protein that could behave as a compensatory protein responding to injuring stimuli.

In vivo, however, no differences in NGB levels in murine models of traumatic brain injury, cerebral malaria, experimental autoimmune encephalitis, hypoxia have been found [8,31,37], insinuating uncertainty that NGB is a stress-inducible protein. Nonetheless, the NGB over-expression confers protection against oxidative stress and enhances cell survival under anoxia and ischemic condition *in vivo* and cultured neurons [13,15,16,39,40]. Because NGB, like other proteins, does not cross cell membranes [41], its direct administration is not a realistic therapeutic strategy. Therefore, substances which increase NGB levels might have a great therapeutic benefit in brain disorders.

The NGB expression can be enhanced experimentally by cobalt, deferoxamine, hemin, and certain short chain fatty acids, valproic and cinnamic acids [42,43]. Valproic acid is a commonly used drug to treat seizures and bipolar mood disorder; however, it is unclear whether NGB induction plays a role in valproic acid neuroprotection. Cinnamic acid is a natural fatty acid obtained from cinnamon oil, but negative result of cinnamic acid in protection against Aβ-induced toxicity in a rat-derived neuronal cell line has been reported [20]. In addition, the high concentration of these substances (0.1 to 1 mM) requested to increase NGB levels poses serious doubts on the possible side effects of these compounds in the brain.

NGB levels are also enhanced by HIF-1 [44], although apparently indirectly, as the NGB promoter lacks HIF-1-binding hypoxia-response elements [45]. More recently, it has been reported that treatment with the vascular endothelial growth factor (VEGF) increases NGB protein expression in cortical neuron cultures, with maximal (about threefold) enhancement at 20 ng/ml of VEGF. In turn, VEGF, via VEGFR2/Flk1 receptors, induces HIF-1α expression which could account for the indirect induction of NGB [42]. Although both VEGF and NGB participate in