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Neuroglobin: an endogenous neuroprotectant

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Abstract

Cerebral hypoxia and ischemia trigger endogenous protective mechanisms that can prevent or limit brain damage. Understanding these mechanisms may lead to new therapeutic strategies for stroke and related disorders. Neuroglobin (Ngb), a recently discovered protein that is distantly related to hemoglobin and myoglobin, is expressed predominantly in brain neurons, and appears to modulate hypoxic-ischemic brain injury. Evidence includes the observations that neuronal hypoxia and cerebral ischemia induce Ngb expression, that enhancing Ngb expression reduces—and knocking down Ngb expression increases—hypoxic neuronal injury in vitro and ischemic cerebral injury in vivo, and that Ngb-overexpressing transgenic mice are resistant to cerebral infarction. However, the mechanisms that underlie hypoxic induction of and neuroprotection by Ngb are still unclear.

Introduction

Globins are O_2-binding heme proteins present in bacteria, protists, fungi, plants and animals [1]. Their functions have diverged widely in evolution, and include binding and transport of O_2, scavenging and detoxification of reactive species including NO, and O_2 sensing. An even wider range of globin functions occurs in nonvertebrates [2,3]. Ancestral globins are thought to have served primarily as scavengers of reactive oxygen and nitrogen species, with a role in O_2 transport developing later [4]. In addition to neuroglobin, five other vertebrate globins are known to exist. The best known of these are hemoglobin, which differs from other vertebrate globins in occurring as a tetramer, is localized to erythrocytes, and transports O_2 between the lungs and other tissues, and myoglobin, which is monomeric and is localized to the cytoplasm of skeletal and cardiac myocytes. More recently discovered globins include cytoglobin, which is widely expressed in vertebrate tissues [5,6], and is distinctive among globins in that it can be detected in both cytoplasm and nucleus [7], and two additional globins that have been identified in vertebrates, but not in mammals: globin E (GbE), which is present in chicken eye [8] and globin X (GbX), which is found in fish and amphibians [9]. Of all the “new” globins, Ngb has received the most attention, partly because of its neuronal localization [10] (Figure 1) and its possible role as an endogenous neuroprotectant [11]. Since Ngb was first described in 2000 [10], much remains unknown about its chemistry and function, although some excellent reviews have appeared [12-14]. In this review we focus specifically on the proposed role of Ngb in protecting central neurons from hypoxic, ischemic and other forms of injury.

Injury-induced Ngb expression

Tissue injury is often associated with the mobilization of molecular and cellular mechanisms that promote repair. Examples include the induction of antiapoptotic proteins and the enhanced

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proliferation of precursor cells to replace cells that are damage or killed. Features of Ngb including its O_2-binding capacity [10] and its relationship to myoglobin, which has been implicated in O_2 transport and in scavenging of toxic compounds like nitric oxide in muscle [15], suggested that Ngb might be a mediator of hypoxic injury-repair coupling in the brain. Such a role implies two phases of action: induction of expression or activation by the inciting injury and the subsequent triggering of protective adaptations (Figure 2). In the first case, Ngb mRNA and protein has now been shown to be induced by hypoxia in cultures of cerebral cortical neurons [11]. Induction is probably dependent on the severity of hypoxia; thus Ngb expression has been shown to be increased by prolonged hypoxia in cell culture [16] and by sustained, but not intermittent, hypoxia in vivo [17]. Enhanced expression of Ngb does not appear to be a universal response to all forms of neuronal injury because at least some insults do not elicit the same response. However, neither is the response to hypoxia unique. Cerebral ischemia is another stimulus to Ngb expression, although findings vary depending on the model used. Focal cerebral ischemia is most clearly associated with Ngb induction [11], perhaps because it involves complete interruption of blood flow and, therefore, profound hypoxia, although focal ischemia did not induce Ngb expression in spontaneously hypertensive rats [18]. Transient global forebrain ischemia increases Ngb expression in gerbil cerebral cortex but not hippocampus [19], and does not alter expression in rat brain [16].

The mechanism for hypoxic induction of Ngb is unknown. Hypoxia-inducible factor-1 (HIF-1) [20,21] is a transcription factor that enhances the expression of a large number of hypoxia-inducible genes, including erythropoietin, vascular endothelial growth factor and glycolytic enzymes. It is, therefore, a candidate intermediary between hypoxia and Ngb induction, but such a connection has not been proven. In favor of HIF-dependence, Ngb, like HIF-1, is induced by cobalt and deferoxamine [11], although these are not entirely HIF-specific [22]. In addition, the 5′-untranslated region of Ngb contains consensus HIF-binding sequences, and the Ngb-related protein, cytoglobin, appears to be HIF-responsive [23]. In cultured HN33 (mouse hippocampal neuron × N18TG2 neuroblastoma) cells, hypoxic induction of Ngb is attenuated by the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) inhibitor PD98059, implicating MAPK signaling pathways [24]. Other mechanisms for the induction of Ngb include that activated by hemin, which is blocked by the protein kinase G inhibitor KT5823 and the soluble guanylate cyclase inhibitor LY83583, is mimicked by treatment with 8-bromo-cyclic guanosine monophosphate (cGMP), and is associated with an increase in cGMP levels, suggesting the involvement of protein kinase G and soluble guanylate cyclase. However, the relevance of this pathway to that activated by hypoxia is uncertain.

If, as suggested below, Ngb is an endogenous neuroprotective agent, how might this protective function be exploited for therapy? As a moderately-sized (~17 kDa) protein that is thought to act intracellularly, Ngb itself is not an obvious therapeutic agent. However, drugs that increase Ngb expression might have such a role. The studies described above suggest that metal chelators like deferoxamine, agents like hemin that stimulate expression of other globins, and compounds that activate MEK, protein kinase G or soluble guanylate cyclase signaling pathways could be worth studying in this regard.

Protection against hypoxia and oxidative injury

The findings reviewed above help assign Ngb to the family of hypoxia-inducible proteins. But is hypoxic induction of Ngb neuroprotective? The first evidence for a neuroprotective role came from studies in which antisense-mediated knockdown of Ngb rendered cortical neuronal cultures more vulnerable to hypoxia, whereas vector-mediated forced overexpression of Ngb in HN33 cells conferred relative protection from hypoxia [11]. Efforts to confer neuroprotection by transduction with a fusion protein in which Ngb is coupled to the human immunodeficiency virus-1 transactivator of transcription (TAT) protein transduction domain

have yielded conflicting results. In one case, treatment with Ngb-TAT failed to rescue a rat retinal ganglion (RGC-5) or human neuroblastoma (SH-SY5Y) cells from combined oxygen and glucose deprivation [25], whereas in another instance, Ngb-TAT-treated cultured rat cortical neurons showed reduced sensitivity to hypoxia [26].

Other studies have shown that cultured human neuroblastoma cells transfected with a Ngb-expressing plasmid are resistant to oxidative injury induced by H_2O_2 [27], which would appear to point to a fairly general or downstream mechanism of protective action, as opposed to a mechanism directed specifically at mitigating cellular hypoxia. In this regard, it has been noted previously that the low (~1 μM) intracellular concentration of Ngb [13] and its comparatively weak O_2-binding affinity (P_{50}≈7.5 torr) under physiological conditions [28] make enhanced O_2 delivery an unlikely modus operandi for neuroprotection. An alternative mechanism is suggested by the finding that Ngb binds not only O_2, but also NO [29], as is also true of Mb, which has been shown to act as a NO scavenger in muscle [30]. Some studies have shown parallelism between the distribution of Ngb and of neuronal NO synthase (nNOS) [31], and NOS expression is increased in several tissues of Ngb-overexpressing transgenic mice [32]. Ngb concentrations are ~1 μM in normal brain [13], and higher in the ischemic penumbra, where NO levels rise from the nM to low-μM range [33]. Under these conditions, autooxidation of Ngb yields $NgbO_2$, which reacts rapidly with NO to form a peroxynitrite ($ONOO^-$)-bound intermediate that decays to yield Ngb and (nontoxic) nitrate [34]. An alternative scheme, in which Ngb reacts with NO directly, has also been proposed [33]. Finally, in addition to heme Fe, reactive thiols in Ngb may also contribute to scavenging $ONOO^-$ [35]. Finally, Ngb inhibits guanine nucleotide dissociation from G-proteins, by binding to $G\alpha_i$ subunits and increasing levels of free Gβγ[36,37], which can activate cell-survival pathways involving phosphatidylinositol-3-kinase [38].

Protection against ischemia

Pure hypoxia (anoxic hypoxia) is an uncommon cause of neurological disease, but hypoxia that occurs as a feature of ischemia (ischemic hypoxia) is frequent. Cerebral ischemia usually results from interruption of blood flow in the extra- or intracranial cerebral circulation and produces the syndrome of stroke, which is characterized by abrupt onset, focal involvement of the brain, circumscribed neurological dysfunction related to the site of involvement, and a tendency for at least partial symptomatic recovery in patients who survive the initial insult. If the protective effect of Ngb against hypoxia is to have clinical implications, stroke is the most likely disorder in which this might be the case.

In support of a role for Ngb in limiting stroke-related brain injury, cerebral infarct size following occlusion of the middle cerebral artery was increased in rats given a Ngb antisense oligodeoxynucleotide by the intracerebroventricular route, and was reduced after intracerebral administration of an adeno-associated virus vector that expressed Ngb [39]. Moreover, similar findings were observed in transgenic mice that overexpressed Ngb [32]. Interestingly these mice, which also overexpressed Ngb in the heart, had smaller myocardial infarcts following coronary artery ligation, implying that the protective effect of Ngb in ischemia must operate through pathways that are also present in non-neuronal cells.

Protection against neurodegeneration

The observation that Ngb can protect cells against non-hypoxic injury [27] raises the possibility that it has a wider endogenous (and, potentially, therapeutic) role than one restricted to hypoxic-ischemic injury. In support of this argument, Ngb-transfected PC12 pheochromocytoma cells show reduced sensitivity to the Alzheimer's disease-related protein, β-amyloid (1−42) [40]. Ngb may have a role in other neurodegenerative disorders as well. As one example, Ngb was

found to be overexpressed in the putamen of a patient with hereditary ferritinopathy, where it may act to mitigate injury triggered by cellular iron overload [41].

Conclusions

Ngb is a relative newcomer among prospective endogenous neuroprotective molecules and therapeutic targets. In fact, we know comparatively little about how Ngb is induced by or protects against neuronal injury, nor about the range of pathological processes in which it may have a role. A judicious summary of what is known would probably include the findings that Ngb expression is induced by neuronal hypoxia, cerebral ischemia and probably other pathophysiological factors, and that Ngb protects neurons subject to profound hypoxia or focal cerebral ischemia, and perhaps also neurodegenerative diseases. The major investigative challenge in this area over the next few years will be to achieve greater understanding of the mechanism through which Ngb protects cells and the spectrum of disease processes against which it is effective.

Acknowledgments

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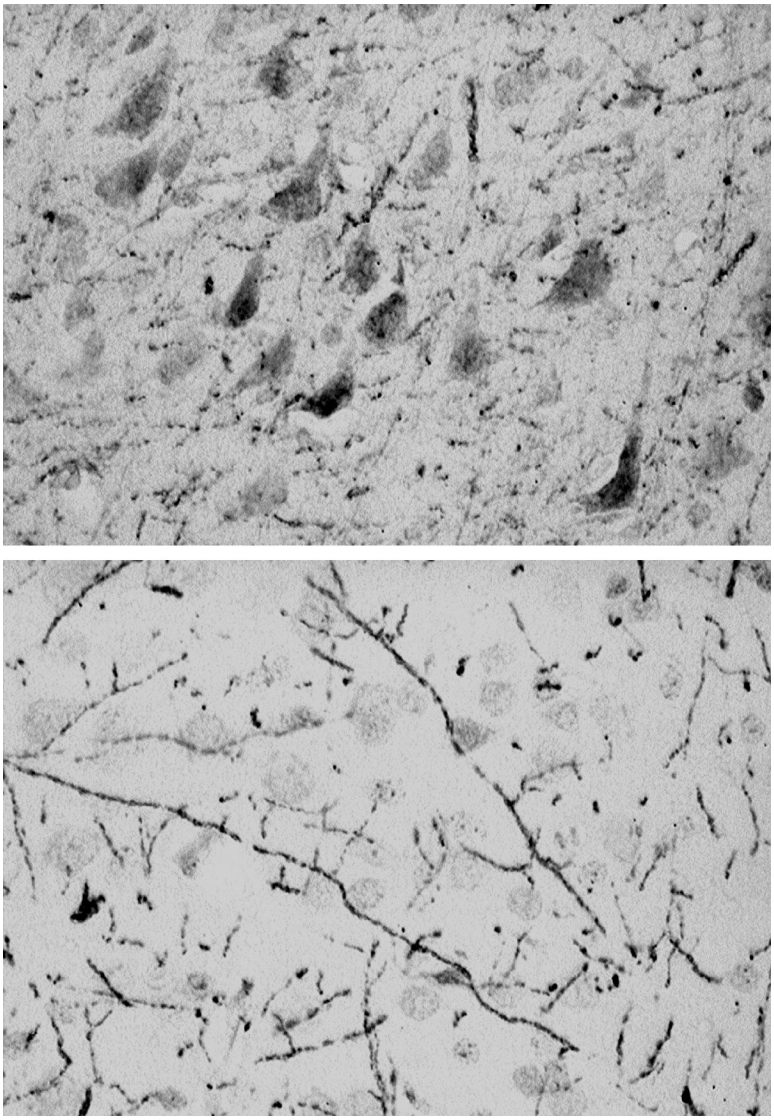

Figure 1.
Neuronal localization of Ngb. Ngb immunoreactivity is associated preferentially with neuronal cell bodies (top) and processes (bottom).

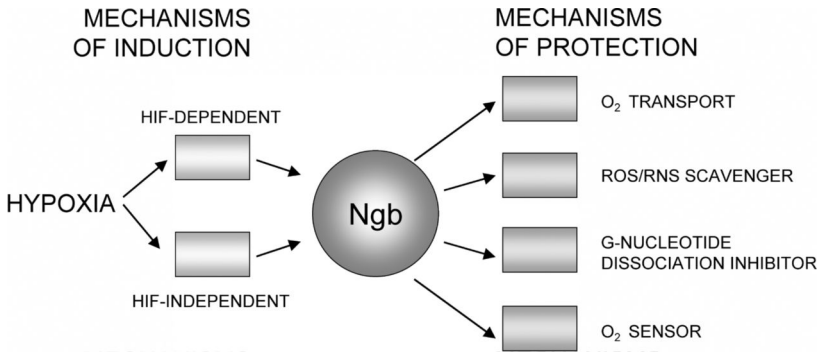

Figure 2.

Ngb expression is inducible by hypoxia and leads to neuroprotection, but the mechanisms for induction and protection are poorly understood. Induction might proceed by HIF-dependent or HIF-independent pathways, or both. Mechanisms proposed to mediate neuroprotective effects of Ngb in hypoxic or ischemic injury include O_2 transport, scavenging of reactive oxygen- (ROS) or nitrogen (RNS)-containing species, enhancing dissociation of Gα- from Gβγ-subunits of guanine nucleotide-binding proteins, and serving as an O_2 sensor that triggers downstream adaptations under hypoxic conditions.