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The Role of Neuroglobin in the Sleep-Wake Cycle

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Abstract

Neuroglobin (Ngb) is a protein expressed in the central and peripherical nervous systems of the vertebrate. The Ngb has different functions in neurons, including regulating O_2 homeostasis, oxidative stress, and as a neuroprotector after ischemia/hypoxia events. The Ngb is a hemoprotein of the globin family, structurally like myoglobin and hemoglobin. Ngb has higher expression in the cortex, hypothalamus, thalamus, brainstem, and cerebellum in mammals. Interestingly, Ngb immunoreactivity oscillates according to the sleep-wake cycle and decreases after 24 hours of sleep deprivation, suggesting that sleep homeostasis regulates Ngb expression. In addition, Ngb expresses in brain areas related to REM sleep regulation. Therefore, in the present review, we discuss the potential role of the Ngb in the sleep-wake regulation of mammals.

Keywords
- sleep deprivation
- REM sleep
- oxidative stress
- neuroprotection

Introduction

Sleep is a state of the conscience regulated by the brain. Evidence shows that different brain areas and neurotransmitters participate in sleep induction and regulation. For example, non-rapid eye movements (NREM) sleep depends on hypothalamic nuclei activity. In contrast, rapid eye movements (REM) sleep is generated by several brainstem nuclei. Both sleep stages are characterized by a specific pattern in electroencephalogram and muscle activity.

NREM sleep is induced by activating the GABAergic neurons from the ventrolateral preoptic (VLPO) and the medial preoptic (MnPO) nucleus. These GABAergic neurons increase firing activity exclusively during an NREM sleep period. Furthermore, the activity of GABAergic neurons inhibits neurons from nuclei that participate in wakefulness induction. In contrast, the orexin (ORX) released by the neurons from the lateral hypothalamus stimulates neurons from the ventral tegmental area (VTA) to release dopamine and induce wakefulness. In addition, the tuberomammillary hypothalamic neurons also are encouraged by ORX to produce histamine release. Finally, ORX stimulates neurons from the locus coeruleus and dorsal raphe nucleus in the brainstem to release noradrenaline and serotonin. In these complex interactions between brain areas and neurotransmitters to induce sleep and wakefulness, diverse intracellular molecules are also involved.

The neural firing rates, synaptic markers, molecular pathways, and gene expression show differential up and down-regulation across brain areas and sleep stages.[1] In this sense, the neuroglobin (Ngb) is a protein expressed in specific brain regions related to the sleep-wake cycle, and its expression change after sleep deprivation. Therefore, in the present review, we discuss the potential role of the Ngb in the sleep-wake regulation of mammals.

Neuroglobin

The Ngb was described in mammals' brains in 2000.[2] It has been reported widely in vertebrates from humans, mice, and

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rats to blowfish and zebrafish, suggesting that Ngb protein is highly conserved through evolution.[3–5] The Ngb is a monomeric globular hemoprotein of about 150 amino acids with a relative molecular mass of 17 kDa. A single copy gene codifies Ngb in all species, and the coding region in the mammalian Ngb gene carries three introns located at B12.2, E11.0, and G7.0. Despite the introns B12.2 and G7.0 being present in other globin genes, the intron E11.0 is unique to Ngb.[6,7] Furthermore, all vertebrate contains this intron in a conserved position within the coding regions except zebrafish, including a fourth intron.[6] The human Ngb gene is located on the long arm of chromosome 14 at position q24.3.[6] Although, Ngb shares about 20% to 25% sequence identity compared to the homologous globins hemoglobin and myoglobin,[3,7] despite the difference, Ngb adopts a 3D folding identical to the rest of the globins, which is the main characteristic of the family (►**Figure 1**).

For example, hemoglobin and myoglobin have six coordination bonds for six ligands; four are nitrogen atoms part of the porphyrin molecule. The fifth bonds bind to the porphyrin with the histidine's nitrogen atom at position 93, while the sixth bond with O_2. In contrast, Ngb forms six bonds, but all of them with O_2.[8] The occupancy of the six-coordinating bonds implies that exogenous ligands must displace the distal histidine to bond to the prosthetic group. Therefore, the mechanism of exogenous ligand binding to Ngb is more complex than the other globins.[8] Thus, Ngb may enhance O_2 to high metabolic neurons and may also be involved in detoxifying reactive oxygen species.[9]

Neuroglobin Functions

Ngb gene and protein are highly conserved throughout evolution.[10] A single copy gene codifies Ngb protein in all species except trout. In the last decade, research on the role that Ngb plays in the brain has intensified. Most studies have been focused on understanding the potential chemical reactions that Ngb regulates and its role as an O_2 transporter in neurons and glial cells.

Experimental evidence indicates that Ngb plays multiple roles in health and disease.[6,11,12] For example, it has been documented that Ngb is expressed at concentrations between 1-100 µM exclusively in the brain, both in neurons and astrocytes and in Müller cells[13,14]; however, Ngb concentration is significantly increased in Müller cells after an ocular injury.[12] Furthermore, biochemical studies support that Ngb can facilitate oxygen transport only if it is at a high concentration. In contrast, low concentrations of Ngb (~1 µM) probably participate only in enzymatic activities and cell signaling.[6,12,15] Furthermore, it has been suggested that high concentrations of Ngb in tissues such as the retina prevent hypoxia and increase O_2 uptake by up to 40%.[16]

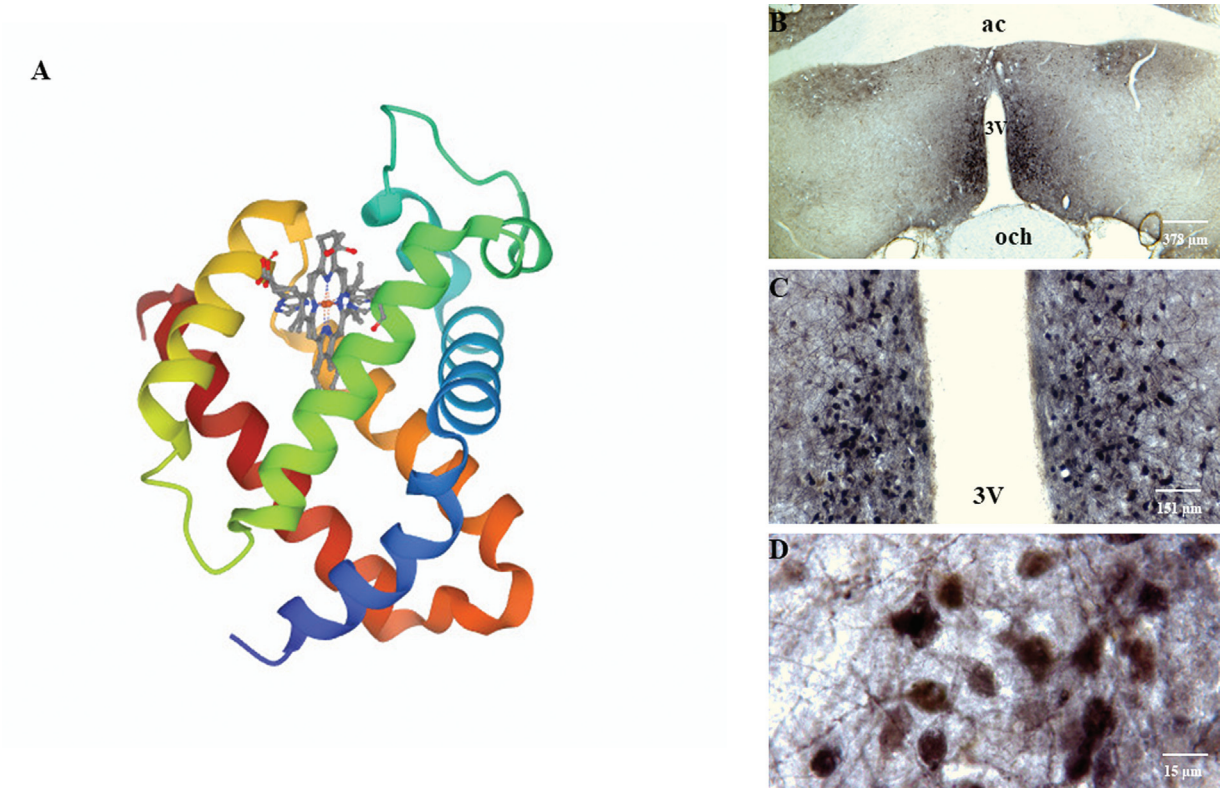

Fig. 1 Crystal structure of murine Neuroglobin (Ngb)[52] and photomicrographic shows basal immunoreactivity to Ngb in the rat's anteroventral periventricular nucleus (avPe). Panel A. Ngb is a hexacoordinated protein that shares 20 to 25% of the amino acid structure with the rest of all globins. Ngb adopts a 3D-folding like other globins, eight alfa-subunits named A-H. Have no external ligands; therefore, any gaseous ligand must compete for binding to the heme iron. Panel B. Coronal section of the avPe illustrates the bilateral immunoreactivity of Ngb cells (4x). Panel C. The density of Ngb positive cells is higher in this brain region (10x). Panel D. Amplification shows the Ngb immunoreactivity in all somas, including the nucleus (100x). 3V third ventricle. Bregma – 0.12 mm. ac anterior commissure, och optic chiasm.

A significant increase in the Ngb immunoreactivity is reported in the cerebellum of animals exposed to moderate concentrations of CO_2 in vivo.[17] In addition, it has been shown that an increase in the expression levels of Ngb in the eye can prevent hypoxia caused by induced ocular hypertension in rats.[18] This result suggests that Ngb could be essential in metabolic adaptation to hypoxic events. Moreover, a decrease in Ngb expression decreases the number and viability of cortical neurons. At the same time, the overexpression of Ngb in the same neuronal type increases the viability of neurons in an in vitro hypoxia model.[19]

Recently, it has been suggested that Ngb regulates the levels of inflammatory cytokines such as Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), Interleukin 1-beta (IL-1B), and Vascular Endothelial Growth Factor (VEGF) and the microglia activation during a hypoxic environment.[20] This effect seems to be related to the regulatory impact of Ngb on the Wnt/β-catenin and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathways, which induce the proteasomal degradation of Disheveled-1.[21]

Although most of the details of the mechanisms through which Ngb exerts its neuroprotective activity are unknown, colocalization studies in cells of the nervous system suggest a direct interaction between Ngb and mitochondria. It has been suggested that Ngb could participate in regulating energy metabolism and apoptosis mechanisms through its interaction with ferric cytochrome c. This antiapoptotic function of ferric cytochrome c requires the participation of Ngb-reducing activity on ferric cytochrome C, which prevents the activation of caspase-9.[22,23]

In the last decade, it has been described that some natural and synthetic compounds with small chemical structures can upregulate the expression of the Ngb due to their ability to cross the blood-brain barrier. For example, in hypoxic cerebral neurons, boosted Ngb expression was observed in cultures treated for 24 h with the iron chelator deferoxamine (Dfx). This effect could be due to Dfx enhanced expression of hypoxia-inducible genes, which are closely related to Ngb expression.[19,24] Besides, valproic and cinnamic acids showed induction of Ngb in vitro. In this sense, valproic acid prompts Ngb expression, like Dfx, whereas cinnamic acid has a more significant effect. However, other short-fatty acids tested did not significantly impact Ngb expression, suggesting that chemical structures and functional groups have a crucial role in this biological effect.[25]

Furthermore, hormones can also influence the upregulation of Ngb. For instance, in the SK-N-BE human neuroblastoma cell line and mouse hippocampal primary neurons, the treatment with 17β-estradiol (E_2) induces an increase in the Ngb protein levels in a time- and dose-dependent manner. Nevertheless, male steroid hormones did not affect Ngb protein expression. Interestingly, polyphenol compounds with estrogen-like properties such as daidzein, genistein, polydatin, biochanin A, and fucosterol provided promising results to induce Ngb upregulation.[26,27] Thus, this evidence suggests exploring new therapeutic approaches for neurodegenerative disorders by establishing in vivo effective doses and adequate treatment periods to prevent adverse effects.

In summary, there is a considerable volume of experimental evidence indicating that exposure to natural or synthetic molecules, hormones or CO_2, the induction of oxidative stress, hypoxia, epilepsy, and ischemia, and even sleep deprivation cause an increase in Ngb levels in the brain and cerebellum that potentially allows it to carry out neuroprotective functions.[28]

The Role of the Neuroglobin in Sleep-Wake Cycle Regulation

The Ngb is expressed in brain nuclei that participate in the sleep-wake cycle. For example, the locus coeruleus nucleus (LC), pedunculopontine tegmental nucleus (PPTg), laterodorsal tegmental nucleus (LDTg), and preoptic area (POA) are brain regions with specific Ngb expression.[29] Also, the neurons from these nuclei modify their firing frequency patterns across the sleep-wake cycle.[30] Recently, we reported reducing the number of Ngb-positive cells in the PPTg, LDTg, and periventricular area (Pe) in rats after sleep deprivation for 24 hours.[31] The corticosterone plasma levels in sleep-deprived rats did not increase compared to non-sleep-deprived rats, suggesting that stress was not responsible for the decrease in Ngb immunoreactivity.[31] Therefore, sleep loss could significantly reduce the Ngb cell-positive cells; perhaps the protein expression is sensitive to disruptions in sleep homeostasis.

In addition, as Ngb exerts antioxidant effects,[32] we evaluated whether sleep deprivation produces oxidative stress in brain tissue since sleep loss induces oxidative stress. However, the results showed that sleep deprivation did not cause oxidative stress in the brain tissue. The above was measured by generating malondialdehyde, a metabolite produced by membrane lipid peroxidation.[31] Altogether, the results support the hypothesis that the expression of Ngb depends on sleep homeostasis because sleep deprivation reduces its immunoreactivity independent of physiological and oxidative stress.

A recent report shows that 6 hours of sleep rebound after 24 hours of sleep deprivation were sufficient to restore the number of Ngb-positive cells in the PPTg and LDTg nuclei. These results suggest that sleep recovery after sleep deprivation promotes Ngb expression.[33] Furthermore, it is well documented that the total time spent in NREM and REM during the initial 6 hours of sleep recovery is increased. Particularly, REM sleep time is increased almost twice after 24-h of sleep deprivation. Moreover, it is reported that EEG slow-wave activity (SWA; mean power density 0.75-4.0 Hz) during NREM sleep is elevated relative to a non-sleep-deprived condition, and the number of brief awakenings is reduced.[34,35] Therefore, it is crucial to characterize whether sleep time after sleep deprivation is associated with Ngb expression.

In addition, in the preliminary study, our group found that the intracerebral administration of the ORX (10μg/5μL, Human orexin, Cat. 06012, Sigma Aldrich, St Louis, MO, USA) in rats results in a significant increase in the total number of the Ngb positive cells in MnPO, Perifornical area, PPTg, and LC. These brain nuclei receive projections

from the lateral hypothalamus and express orexin receptors.[36] ORX is a peptide synthesized by neurons located through the medial-lateral extent of the hypothalamus and responsible for inducing wakefulness.[36] Furthermore, prolonged wakefulness causes cellular damage.[37,38] This cellular damage is related to neurodegenerative disease development.[39] In this sense, in vitro studies suggest that cytosolic Ngb could react with mitochondrial cytochrome c, interfering with apoptotic pathways to prevent cellular damage.[23,28,40] Therefore, Ngb could participate as an endogenous neuroprotector in situations of sleep loss and prolonged shift work. For example, patients with obstructive sleep apnea (OSA) and that who suffer sleep fragmentation during the night report higher serum levels of Ngb, suggesting that Ngb has a protective role in conditions of low oxygen levels.[41]

In addition, lower Ngb expression after orexin administration was observed in the LDTg nucleus. Several studies in cats and rats show that the administration of ORX into the pons, particularly in the LDTg nucleus, promotes wakefulness and suppresses REM sleep.[42,43] Furthermore, an optogenetic study reported that activating cholinergic neurons in the PPTg during the NREM sleep period induces REM sleep. However, this effect was less evident when stimulated LTDg neurons.[44] Together these results suggest that PPTg could participate more actively than the LDTg nucleus in controlling the onset of REM sleep. These results also support the hypothesis that Ngb is increased to mediate the metabolic rate induced by sustained neuronal activity (►**Figure 2**).

As aforementioned, sleep loss associated with OSA disorder increase Ngb serum levels. The OSA is a condition that generates intermittent hypoxia (IH), sleep disruption, and increased apoptotic processes due to oxidative stress. Interestingly, plasma levels of Ngb increase in proportion to the severity of the OSA.[41] Furthermore, Ngb over-expression in IH animal models protects against lipid peroxidation.[45,46] In a murine model of ischemia-reperfusion, Ngb positive cells notably increased 24 h post-reperfusion in the cerebral cortex and remained higher than controls 48 h after ischemia-reperfusion.[46] These findings provide evidence for the neuroprotective role of Ngb during cellular stress.

Sleep is also associated with neuroprotective functions since sleep loss is a risk factor for developing Alzheimer's and other neurological and psychiatric disorders. Furthermore, Ngb is also related to neurodegenerative diseases such as Alzheimer's.[47–49] In this sense, it is plausible that Ngb acts as the first line of defense in developing neurodegenerative pathologies. Therefore, even when it sounds logical, it is necessary to investigate whether chronic sleep restriction may affect Ngb expression and if the reduction of the Ngb induces the neurodegenerative disease's progress.

Ngb is highly expressed in neurons of the suprachiasmatic nucleus (SCN) in the hypothalamus that serves as the master clock in the mammal's brain. SCN neurons co-express Ngb and PER-1, a clock gene involved in the circadian regulation and maintenance of SCN activity.[50] SCN receives a monosynaptic input from the retina, where Ngb is highly expressed. Interestingly, Ngb mRNA expression in SCN neurons of mice increases during the light period; meanwhile, during the dark period decreases, showing an evident circadian pattern.[51] Then, Ngb-deficient mice disrupt the SCN light responses and increase the expression of PER1 after a light stimulus during the dark period.[51] It also correlated with

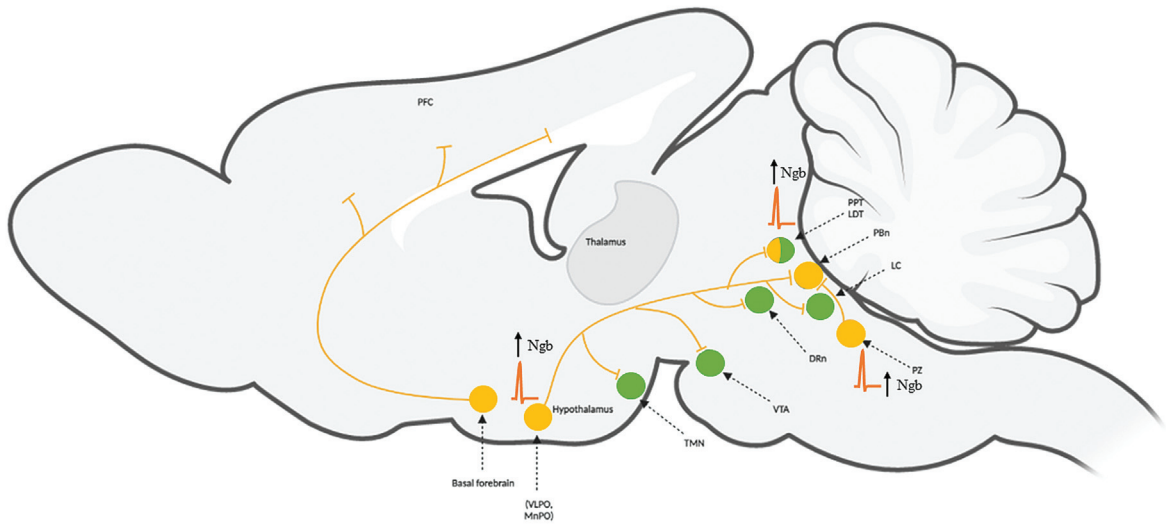

Fig. 2 Neuronal pathways that regulate sleep. The ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (MnPO) send inhibitory projections to the brain nucleus in the hypothalamus and brainstem involved in wake to induce sleep. The activation of the neurons hypothetically induces neuroglobin (Ngb) expression in the nuclei related to sleep. Ngb can participate in the control of metabolic rate induced by oxidative stress.
Abbreviations: TMN, Tuberomammillary nucleus; VTA, ventral tegmental area; DRn, dorsal raphe nucleus; PBn, parabrachial nucleus; PZ, parafacial zone.

preliminary results of our laboratory that show an increase in the number of Ngb-positive cells during sleep (light phase) and a decrease during the wake phase (dark phase). Since sleep is regulated in a circadian fashion directly from SCN, it could be one of the main signals for Ngb synthesis and regulation during sleep.

Conclusions

The evidence discussed in the present review suggests that Ngb is susceptible to response to sleep loss and sleep rebound restored. Potentially, Ngb could participate as an endogenous neuroprotector in situations of sleep loss and prolonged shift work. These results also support the hypothesis that Ngb is increased to mediate the metabolic rate induced by sustained neuronal activity. However, more studies are necessary to understand the role of the Ngb in sleep and other behaviors and the consequences of its deficiencies.

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Conflict of Interest Statement
None declared.

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