

Review

Neuroglobin and neuroprotection: the role of natural and synthetic compounds in neuroglobin pharmacological induction

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Abstract

Neuroglobin (Ngb) is a 17 kDa monomeric hexa-coordinated heme protein belonging to the globin family. Ngb is mainly expressed in neurons of the central and peripheral nervous system, although moderate levels of Ngb have been detected in non-nervous tissues. In the past decade, Ngb has been studied for its neuroprotective role in a large number of neurological disorders such as Alzheimer's disease, Huntington's disease, brain ischemia and hypoxia. This review discusses and summarizes the natural compounds and the small synthetic molecules capable of modulating Ngb expression that exhibits a protective role against various neurodegenerative diseases.

Key Words: Neuroglobin; neuroglobin pharmacological induction; neuroprotection; neurodegenerative diseases; natural compounds; synthetic small molecules; Alzheimer's disease; Huntington's disease

Introduction

Globins are heme-proteins that binding oxygen and are present in all living organisms. They play an essential role in bacteria, plant, fungi and animal respiration and oxidative energy production. For several years, hemoglobin (Hb) and myoglobin (Mb) were the only two globins identified in vertebrates (Pesce et al., 2002). Hb is found in the red blood cells, it carries the oxygen (O₂) molecule from the lungs to all tissues and it contributes to control the pH of body fluids. Mb is localized in the skeletal and cardiac muscular tissue, and it is involved in the storage and increase in O₂ transfer to the mitochondria. In addition, Mb is implicated in the detoxification of nitric oxygen and reactive oxygen species (Rassaf et al., 2014).

In 2000, neuroglobin (Ngb), a third type of globin, was discovered (Burmester et al., 2000). Ngb is a heme-protein that is highly conserved throughout evolution, and human and mouse Ngb have a sequence identity higher than 90% (Ascenzi et al., 2016). Ngb is widely expressed in central and peripheral nervous systems, and several studies suggest that it has a central role in neuroprotection. This review presents the natural and synthetic compounds able to modulate Ngb expression, and which show therefore a possible protective role against various neurodegenerative diseases.

Search Strategy

For the present review, we searched the literature using keywords such as Neuroglobin up-regulation, Neuroglobin pharmacological inductors, natural and synthetic compounds in neurodegeneration, on PubMed, Google Scholar and Sci-Finder. In addition, we also used modifications of the above main keywords to thoroughly search the literature.

Neuroglobin Structure and Functions

Ngb, a monomeric 151 amino-acid protein, is a nerve globin family member widely present in vertebrates and largely expressed in neurons of the central and peripheral nervous system and in endocrine tissue (Burmester et al., 2000). Globins are globular metalloproteins that share a common tertiary structure characterized by six or four α -helices (3/3 or 2/2-fold symmetry, respectively) that make a sandwich around a heme group. They reversibly bind O₂ via an iron-containing porphyrin ring.

Human Ngb is distributed widely in the human body, including the hippocampus, thalamus, hypothalamus, cerebral cortex, cerebellum, organs with endocrine function and retinal cells. It has been found that in cerebral hypoxia and ischemia injury Ngb is up-regulated suggesting a neuroprotective role in brain disorders (Li et al., 2010). The Ngb concentration is different across the various human brain sections (Van Acker et al., 2019). It has been reported that Ngb overexpression is related to cytoprotective effects on neurons, anti-apoptotic features on nerve tissue and protection against oxidative stress (Ascenzi et al., 2016; Luyckx et al., 2019). Despite the numerous studies performed, the exact functions of Ngb are difficult to define.

As a globin, the main physiological functions of Ngb are binding and transport of O₂, scavenging and detoxification of reactive species (including nitric oxygen, carbon monoxide, or hydrogen sulfide), as well as O₂ sensing (Burmester and Hankeln, 2014; Ascenzi et al., 2016; Anna Bilska-Wilkosz et al., 2017). Ngb has the common globin structure consisting of eight α -helices denoted A–H, with the heme prosthetic group located between helices E and F.

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While in Hb and Mb proteins the heme iron (heme-Fe) is pentacoordinate, the heme-Fe of Ngb is hexacoordinated by four pyrrole N atoms within the heme plane, the distal His(E7)64 side chain and the proximal His(F8)96 residue (**Figure 1**). The comparison of the high resolution Ngb crystal structure PDB code: 4MPM (1.74 Å) with the other previous deposited structures (human and murine, wild type (wt) and mutant) suggest that the flexibility of the loop between the helices C and D is crucial for the Ngb ability to bind and stabilize exogenous gaseous ligands (Guimarães et al., 2014).

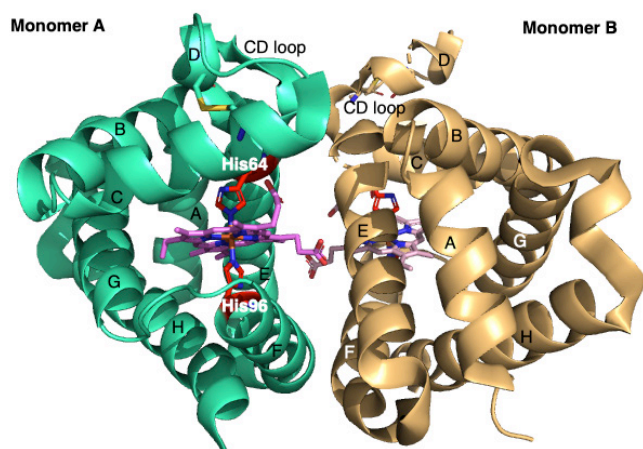


Figure 1 | X-ray crystal structure of human Ngb (PDB code: 4MPM).

Graphical representation of the two Ngb monomers (A and B) in the asymmetric unit, light green and light orange respectively. A, B, E, F, G and H α -helices, forming the classical 3/3 sandwich are labelled in both monomers. The hexacoordinated heme group is coloured in violet (Monomer A) and light pink (Monomer B), His64 and His96 are coloured in red. In both monomers, the disulfide bridges located in the CD loop/D helix are labelled. CD loop: The loop between the helices C and D; Ngb: neuroglobin.

Recently, it has been reported that Ngb, as well as Cystatin C, Apolipoprotein A1 and Transthyretin, has a potential protective role in Alzheimer's disease (AD; Ciccone et al., 2018, 2020a; Van Acker et al., 2019). Previously, *in vitro* studies showed that Ngb reduced amyloid- β ($A\beta$) protein toxicity in mouse neurons and pheochromocytoma cell lines suggesting that its antioxidant properties could protect against AD (Khan et al., 2007; Li et al., 2008). Moreover, *in vivo* investigations showed that Ngb expression increases in AD (Chen et al., 2012), even if the precise mechanisms of Ngb protection against AD remains largely unknown. Experiments, led on human brain of post-mortem AD patients, showed an up-regulation of Ngb expression in hippocampal tissue during early and moderate stages of AD, while the Ngb concentration decreased to control level in advanced AD (Sun et al., 2013). In another study, it has been hypothesized that Ngb through its activation by PI3K/AKT pathway inhibits caspase activity supporting cell survival, an alternative possible strategy to prevent the progression of AD (Li et al., 2016).

The potential neuroprotective function of Ngb has been also investigated in Huntington's disease (HD), an inherited rare diseases related to a progressive degeneration of neurons in the brain (McColgan and Tabrizi, 2018). It has been reported that huntingtin (Htt) and Ngb take part in neuroprotective axis triggered by the hormone 17 β -estradiol (E2) protecting nerve cells from apoptosis (Nuzzo et al., 2017), this synergic pathway fails when huntingtin is mutated. Starting from the idea that neuronal Ngb expression and HD are strictly related, colocalization and interaction between Ngb and Htt were studied in wt- and R6/2 mice models of HD. The result showed a colocalization between Ngb and Htt in wt-mice but not in R6/2 HD models, therefore it has been hypothesized that the Ngb and Htt interaction is mediated by other partners (Cardinale et al., 2018).

The Ngb neuroprotective role is probably related to a synergic mechanism which involves improving mitochondria function, decreasing the secretion of reactive oxygen species and nitric oxygen and inhibiting the intrinsic pathway of cell death (Khan et al., 2007; Li et al., 2008; Jin et al., 2009; Raychaudhuri et al., 2010).

Moreover, it has been suggested that using natural and chemical molecules to induce the Ngb expression in different pathologies, such as ischemia, hypoxia, AD and HD, could be a new therapeutic approach against neurodegenerative diseases (Yu et al., 2009; Jin et al., 2011). Here we summarize the natural and small synthetic molecules that have been studied for their ability to modulate Ngb expression.

Pharmacological Induction of Ngb: Natural and Synthetic Compounds

Various diseases and injuries that affect the central nervous system can be attenuated by Ngb overexpression (Greenberg et al., 2008). Unfortunately, the direct administration of recombinant Ngb in therapy is not suitable because this protein is intracellular and it does not pass through cellular membranes, except in zebrafish (Watanabe and Wakasugi, 2008). However, it has been shown that the use of certain small molecules (natural and synthetic), which are able to cross the blood brain barrier (BBB), can induce the up-regulation of Ngb leading to an improvement in outcome of brain injuries and neurodegenerative disorders in both *in vitro* and *in vivo* experiments (Yu et al., 2012).

In **Additional Table 1**, we list the natural and synthetic molecules able to modulate Ngb, reported in the literature to the best of our knowledge. The compounds have been grouped according to their chemical structure or function into six classes: iron chelators, natural hormones and derivatives, plant derivatives, short fatty acids, nonsteroidal anti-inflammatory drugs, and antidiabetics. Within each class, we specify the origin, natural or synthetic, of the reported compounds.

Furthermore, for the discussed compounds, the structure, the experimental models and the principal clinical uses have been reported in **Additional Table 1**.

Iron chelators

The first molecule identified as an Ngb inducer was deferoxamine (Dfx; **Additional Table 1**), a natural chelating BBB-crossing molecule which is synthesized by *Streptomyces pilosus* in iron-limiting conditions (Ellenberg et al., 1990; Sun et al., 2001). Dfx is a drug that finds application in the treatment or prevention of iron overload related to thalassemia, and it is a chelating agent able to complex iron favouring its excretion (Olivieri and Brittenham, 1997). It has been shown that Dfx increases the expression of hypoxia inducible gene factor 1, one of the principal transcriptional factors for hypoxia signalling, connected to Ngb expression (Sun et al., 2001).

The literature reports that hemin (**Additional Table 1**), a porphyrin containing a ferric iron ion with a chloride ligand, can induce the expression of Hb and Mb (Rutherford et al., 1979; Graber and Woodworth, 1986). Starting from the structural and functional similarity of Ngb with Hb and Mb, it was suggested that hemin can also induce Ngb expression. This hypothesis was confirmed by an *in vitro* experiment where the HN33 cells (somatic cell fusion of mouse hippocampal neuron and N18TG2 neuroblastoma) treated with hemin showed Ngb up-regulation. It has been hypothesized that, the overexpression mechanism could be regulated by the soluble guanylate cyclase-protein kinase G pathway (Zhu et al., 2002). Moreover, in a recent study, it has been shown that intravenous administration of hemin, in animal models of

retinitis pigmentosa, significantly increases the Ngf expression in retina reducing its degeneration (Tao et al., 2018).

Hormones and derivatives

Several studies have suggested that the estrogen hormones have an important physiological role in the brain and protect from neurodegenerative disorders (Vasudevan and Pfaff, 2008). It was highlighted that women in pre or menopause periods are more sensitive to develop AD (Uddin et al., 2020). Particularly, E2 decreased A β and glutamate toxicities (Xu et al., 1998). The concept that endogenous modulators such as hormones could also have a role in Ngf regulation levels, prompted scientists to evaluate estrogens in several insults hypothesizing that Ngf can participate in the E2 signalling induction pathway.

The first experimental evidence of the interaction between Ngf and E2 was reported by De Marinis et al. (2010), as shown in **Additional Table 1**. They demonstrated that E2 increased the Ngf levels up to 300% in both the SK-N-BE human neuroblastoma cell line and mouse hippocampal neurons, showing that E2 can act as an endogenous modulator of Ngf expression (De Marinis et al., 2010, 2013b). The SK-N-BE cells, containing high estrogen receptor (ER) β and low ER α levels, were stimulated with either the specific ER α agonist 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol or the appropriate ER β agonist 2,3-bis(4-hydroxyphenyl)propionitrile (DPN) to evaluate the role of each ER subtype in the E2-induced Ngf pathway. Only DPN simulated the E2 effect on Ngf levels, confirming the role of ER β in Ngf induction (De Marinis et al., 2010; **Additional Table 1**).

Also, the upregulation of the Ngf expression induced by E2, in astrocytes, may be related to a mechanism involving estrogen receptor ER β (De Marinis et al., 2013a).

However, E2 can also bind to the ER α receptor subtype inducing a cancer survival effect and Ngf expression. Experimental data reported that ER α /ER β ratio is higher in non-neuronal cancers (such as breast and liver) than in normal tissue (Acconcia et al., 2005). Further studies showed that E2 induced Ngf up-regulation both hepatoma and breast adenocarcinoma (MCF-7) cells, which express the ER α receptor subtype (Fiocchetti et al., 2014).

In the literature, controversial results have been reported regarding testosterone, a gonadal hormone. Testosterone alone does not increase the Ngf expression, either in SK-N-BE cells nor mouse in hippocampal neurons (De Marinis et al., 2010), but in another study, it was found that, when T98G cells (human astrocyte cell model) are exposed to glucose deprivation, testosterone induces Ngf production. The latter result suggests that testosterone can regulate the levels of neuroprotective proteins under cellular damaging conditions (Toro-Urrego et al., 2016; **Additional Table 1**).

Fucosterol (Fuc) is a phytosterol largely present in brown marine algae and shows several biological effects (anticancer, antidiabetic, antioxidant, antifungal, antihistaminic, anticholinergic, antiadipogenic, etc.) (Abdul et al., 2016). In addition to the previously mentioned endogen hormones, Fuc was studied as a potential Ngf regulator (**Additional Table 1**). Human neuroblastoma cell line (SH-SY5Y cells) treated with Fuc showed a remarkable increase of Ngf messenger RNA (mRNA) levels. Moreover, if the SH-SY5Y cells were pre-treated with Fuc before the treatment with A β -induced toxicity, the mRNA levels of Ngf increased greatly showing that the neuroprotective effect of Fuc is related to Ngf up-regulation (Gan et al., 2019).

Tibolone (Tib) is a synthetic BBB-crossing steroid hormone that is used in menopause hormone therapy (Kuhl, 2005) and in the prevention of osteoporosis (Ettinger, 2007; Biglia et al., 2010).

Several results, from experimental studies and clinical trials, have suggested that Tib has a neuroprotective effect in CNS (Pinto-Almazán et al., 2017). Tib, such as E2, has a protective effect on astrocytes due to its interaction with ER β and its ability to upregulate Ngf mRNA (Avila-Rodriguez et al., 2016; **Additional Table 1**). Furthermore, it has been shown that Tib has a beneficial effect on BV-2 cells (used as microglia cell model) against inflammation and oxidative stress induced by palmitic acid. The mechanisms by which Tib on its biological activities include the activation of estrogenic pathway via ER β and the modulation of Ngf levels as well as, reducing mitochondrial damage and inflammation (Hidalgo-Lanusca et al., 2018).

Thyroid hormones have an important role in brain development because they are fundamental for growth and differentiation. An *in vivo* study evaluated the variation of the levels of two globins located in the brain: Ngf and cytoglobin. It was found that, when rats were treated with high doses of triiodothyronine (T3), both proteins were overexpressed (Oliveira et al., 2015; **Additional Table 1**). Even if this study demonstrated an increase in gene and protein expression of the two globins in the brain, further investigations need to be done to elucidate the precise mechanism induced by T3 hormone. Regarding Ngf, one hypothesis is that T3 could upregulate Ngf expression indirectly acting on the hypoxia inducible gene factor 1 (Oliveira et al., 2015).

In addition to the mentioned hormones, erythropoietin also plays a neuroprotective role inducing Ngf up-regulation (Milano and Collomp, 2005). Recently, an *in vivo* study showed that erythropoietin can stimulate Ngf protein expression in the Mongolian gerbil brain (Gao et al., 2011).

As it is well-known, neuronal response to hypoxic or ischemic injury induces the expression of neuroprotective proteins such as Vascular endothelial growth factor (VEGF). A study, conducted in neuron-enriched murine cerebrocortical cultures, demonstrated that there was a positive correlation between VEGF and Ngf. VEGF upregulated Ngf expression and Ngf downregulated VEGF expression (Jin et al., 2012).

Plant derivatives

Polyphenols are natural compounds containing phenolic groups. They constitute a large area of research in therapies against degenerative diseases including neurodegeneration and cancer (Ortore et al., 2016; Renaud and Martinoli, 2019; Di Meo et al., 2020). The biological effects have been attributed to their antioxidant capacities, their protective capabilities on microcirculation and their estrogen-like anti-inflammatory actions (Poschner et al., 2019).

Naringenin (Nar) is a flavonoid largely studied for its neuroprotective potential in several degenerative pathologies (Chen et al., 2019a; Ciccone et al., 2020b). Conversely, it was found that Nar induced the up-regulation of Ngf in SK-N-BE cells interacting with the ER β subtype (De Marinis et al., 2010; **Additional Table 1**). Interestingly, in another study the Nar effect was investigated in MCF-7 cell line and the result showed that it did not modify the Ngf protein level (**Additional Table 1**). Moreover, if E2 was co-administered with Nar, the latter antagonized E2 inducing Ngf upregulation (Cipolletti et al., 2019).

A group of 35 natural compounds were tested using cell based reporter systems (mouse and human) for identification of new Ngf-upregulating molecules. Daidzein (Dzn), genistein, polydatin, biochanin A and especially formononetin were found to result in an increase in Ngf mRNA expression both in mouse and human primary neurons (Sun et al., 2001; **Additional Table 1**). Notably, among the five natural inducers, biochanin A, formononetin genistein and Dzn are considered phytoestrogens. They are structurally and

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functionally similar to estrogen hormones, such as E2, which was previously mentioned as an endogen up-regulator of Ngf gene expression in neurons (De Marinis et al., 2010, 2013b). Among the five new Ngf activators, formononetin displays the highest capability to induce Ngf overexpression. Further investigation has shown that formononetin could induce Ngf up-regulation through activation of cAMP response element-binding protein (Liu et al., 2016).

Recently, Dzn and its metabolites were tested in ER α positive breast cancer cells (MCF-7 and T47D) in order to evaluate the possible correlation between ER α activation and Ngf levels (Montalesi et al., 2020; **Additional Table 1**). Contrary to E2, which prompts Ngf up-regulation, the treatment of MCF-7 and T47D cells with Dzn (1–10 μ M) reduced Ngf below baseline levels rendering breast cancer cells more sensitive to paclitaxel treatment. The same effect is observed with Dzn-4'-sulfate metabolite, administered at lower concentrations than daidzein (0.1–1 μ M) (Montalesi et al., 2020). The other metabolites studied, Equol (Eq), Dzn-7-sulfate (D7S), D-4',7-disulfate and O-desmethylangolesin had an E2 like effect, increasing Ngf levels proportionally to the concentration used. Precisely, D-4',7-disulfate (DDS) and O-desmethylangolesin induced Ngf up-regulation at low concentrations, while they showed no effect at high concentrations. The complexity of obtained results suggest that Dzn and its metabolites probably trigger different signal transduction pathways (Montalesi et al., 2020).

Among natural polyphenols, resveratrol (Res) has been largely studied for its numerous potential biological activities including: anti-oxidation, anti-inflammation, anti-cancer, cardioprotection, neuroprotection and estrogenic/antiestrogenic functions (Chen et al., 2019b; Xiao et al., 2019; Ahmadi and Ebrahimzadeh, 2020). Studies have reported that neuronal derivatives SK-N-BE cells, in which ER β expression is higher than ER α , treated with Res display Ngf up-regulation. This effect persists also when these cells are co-stimulated with E2, showing that Res is an E2 agonist in presence of ER β and it is an E2 antagonist in presence of ER α (Cipolletti et al., 2019). Due to several reports present in literature suggesting that Ngf also has a protective role in cancer cells (Emara et al., 2010; Ascenzi et al., 2016), Res was tested in two ER α -positive breast cell lines (MCF-7 and T47D). In both experiments Res, acting on E2-induced ER α signalling pathway, decreased Ngf levels rendering cancer cells less resistant to chemotherapeutic drug paclitaxel (Cipolletti et al., 2019).

In addition to Res, quercetin and 8-prenyl-naringenin were studied as modulators of Ngf levels in ER α positive breast cancer cells (**Additional Table 1**). Both of them show Ngf overexpression suggesting that their role as E2 agonists on ER α , without interfering with the E2 effect, if the latter is co-administered (Cipolletti et al., 2019).

In summary, Res, Nar, genistein and Dzn act as antiestrogenic molecules in presence of ER α (i.e., breast cancer cells), and as a consequence, they reduce E2-induced breast cancer cell proliferation. In line with this, these polyphenols reduce the Ngf levels rendering cancer cells more prone to cell death. Note that, different metabolites of these compounds (i.e., daidzein metabolites) act differently from their precursors (i.e., daidzein).

On the other hand, Res and Nar (but also genistein) act as estrogenic molecules in the presence of ER β subtypes (mainly expressed in neuronal derived cells), increasing the Ngf levels and protecting neurons from oxidative stress injury.

In order to discover new natural compounds capable of inducing Ngf activation, 457 Indonesian phytochemicals have been evaluated *in silico* using the molecular docking technique. It has been reported that Ngf can be activated by 14-3-3 protein, but during hypoxia the 14-3-3 protein shift into

the neuron nuclei reducing the Ngf neuroprotective function (Jayaraman et al., 2011). A virtual screening study was used to identify new compounds that mimic 14-3-3 protein in the Ngf activation process. Among all the screened compounds, two phytochemicals: Miraxanthin-III and Strigol (**Additional Table 1**) gave good binding scores and similar conformations compared to selected 14-3-3 residues, suggesting that both of these natural products can be considered potential Ngf activators (Pangestu et al., 2018).

Short-chain fatty acid derivatives

Starting from previous results showing that short-chain fatty acid derivatives induced fetal globin expression (Pace et al., 2002), the cinnamic, valproic, butyric, levulinic and succinic acids were studied in HN33 cells in order to investigate if they can act as regulators of Ngf levels (Jin et al., 2011). Among the fatty acids screened, only cinnamic and valproic acids exhibited relevant induction of Ngf (Jin et al., 2011; **Additional Table 1**). Valproic acid is a synthetic compound present in the formulation of several anticonvulsant drugs, while cinnamic acid is a natural product extracted from cinnamic oil. The mechanism by which these fatty acids can up-regulate the Ngf expression is not well understood. Studies suggested that the neuroprotection benefit induced by valproic acid is attributed to histone deacetylase inhibition; however this biological activity seems not related to the induction of Ngf expression (Jin et al., 2011).

Moreover, due to the anticonvulsant activity of valproic acid other anticonvulsants were tested, but none of them showed Ngf induction (Jin et al., 2011).

Nonsteroidal anti-inflammatory drugs

As mentioned above, Ngf is a neuroprotective protein implicated in AD. It supports neuronal cell survival *in vitro* and *in vivo* protecting the brain from damage induced by AD progression (Khan et al., 2007; Li et al., 2008; Brittain et al., 2010). In a recent study, a co-drug composed of ibuprofen and α -lipoic acid (IBU-LA) was investigated as a potential inducer of Ngf expression in a rat brain model of AD (Zara et al., 2013; **Additional Table 1**). Both IBU and LA have already shown protective action in AD (Weggen et al., 2001; Hager et al., 2007). The IBU-LA co-drug is highly resistant to enzymatic degradation; it crosses the BBB favoring IBU and LA delivery directly to neurons. The results obtained show that the subcutaneous administration of IBU-LA guaranteed a high level of Ngf in the rat brain of the AD model compared to its control (Zara et al., 2013).

Antidiabetic drug

Metformin (Met) is an oral antidiabetic drug largely used in the treatment for type-2 diabetes (Thomas and Gregg, 2017). Recently, Met has been investigated for its potential role in neurodegenerative diseases (Rotermund et al., 2018). In a study performed on adult Wistar rats, the animals were treated with alcohol to induce neurotoxicity and after Met was orally administered for several days (**Additional Table 1**). The result shows that, in response to brain injury induced by alcohol, Met increased Ngf expression in the frontal lobe (Bonea et al., 2020). Moreover, Ngf up-regulation was also found in rats treated only with Met, confirming the antioxidant and protective biological functions of Met (Bonea et al., 2020).

Conclusion

Ngf is a globin discovered 20 years ago. Since then, Ngf has largely been studied for its several biological functions. A considerable amount of experimental evidence shows that Ngf can be considered an endogenous neuroprotective molecule, because its upregulation ameliorates the critical profile that arises after ischemic strokes and brain injuries in

general. Moreover, several studies have revealed that Ngb has a protective role in neurodegenerative disorders such as AD and HD, where the decreased expression of Ngb has been associated with increased risk of the pathologies' onset and evolution.

In this review, we collate and discuss the materials available on a number of natural and synthetic compounds, discovered so far, that can act as potential Ngb pharmacological inductors with the aim to summarize all of the principal information in a concise but exhaustive manner.

This overview highlights the chemical structures that can inspire future studies for the design of new molecules able to modulate Ngb level.

The endogenous neuroprotection induced by Ngb overexpression could represent a winning therapeutic strategy. Furthermore, the pharmacological induction of Ngb by natural and synthetic molecules could be useful in the treatment of neurodegenerative diseases that, to date, are lacking in effective therapies.

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Additional file:

Additional Table 1: Natural and synthetic compounds as Ngb pharmacological modulators.

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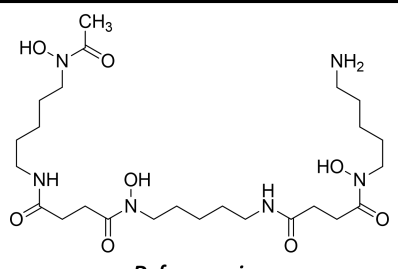
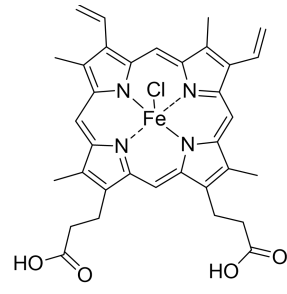
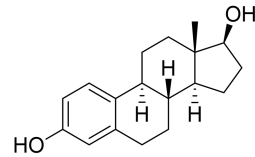
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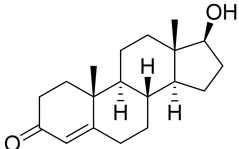
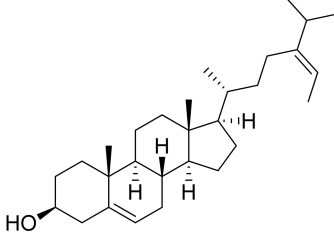
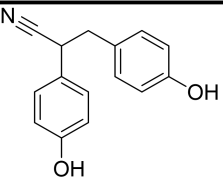
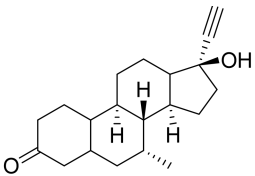
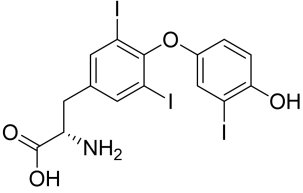
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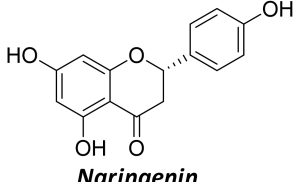
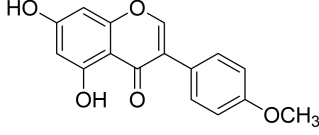
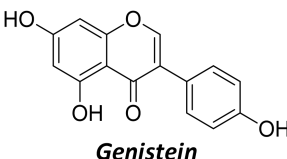
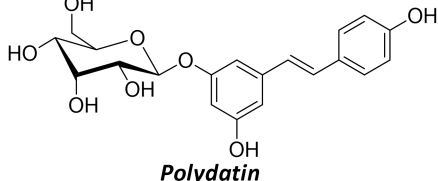
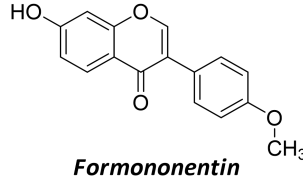

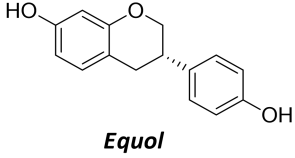
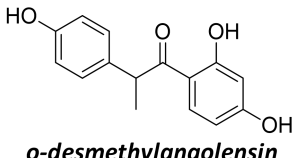
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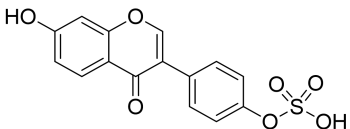
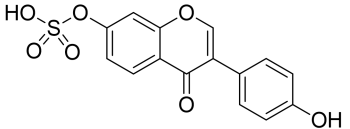
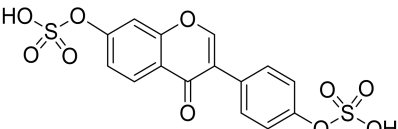
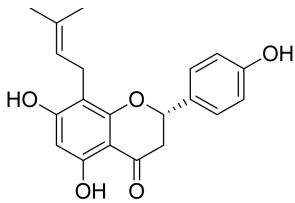
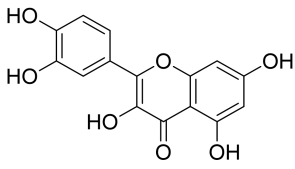
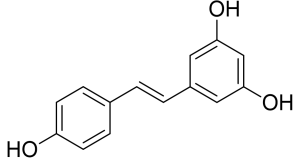
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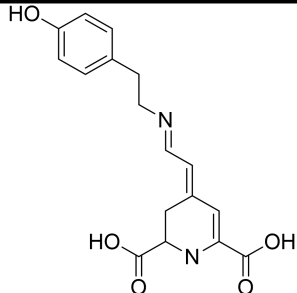
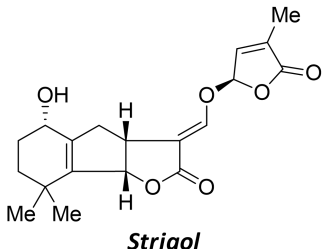
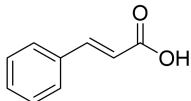
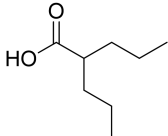
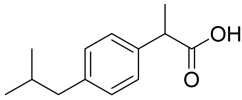
Additional Table 1 Natural and synthetic compounds as Ngb pharmacological modulators

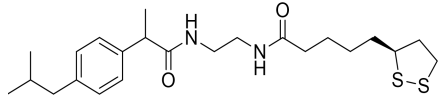
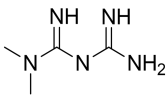
Molecule	Model			Clinical use	Reference
	Primary cultured neurons	<i>In vivo</i>	Non-neuronal cultured cells		
Iron chelators					
<i>Synthetic</i>					
 Deferoxamine	SK-N-BE cells mRNA ↑ Ngb ↑			Iron overload	Sun et al. (2001)
<i>Natural</i>					
 Hemin	HN33 cells mRNA ↑ Ngb ↑	C57BL/6 mice (Retina) mRNA ↑ Ngb ↑		Porphyria	Zhu et al. (2002); Tao et al. (2018)
Hormones and derivatives					
Steroids and analogues					
<i>Naturals</i>					
 17-beta-estradiol	SK-N-BE cells, astrocytes mRNA ↑ Ngb ↑		MCF-7, HepG2 mRNA ↑ Ngb ↑	Menopause hormone therapy	De Marinis et al. (2010); Fiocchetti et al. (2014); De Marinis et al. (2013a)

 Testosterone	T98G cells mRNA n.d. Ngb ↑	Hormonal deficiency	Toro-Urrego et al. (2016)
 Fucosterol	SH-SY5Y cells mRNA ↑ Ngb n.d.	n.d.	Gan et al. (2019)
Synthetic			
 Diarylpropionitrile	SN-K-BE mRNA ↑ Ngb ↑	n.d.	De Marinis et al. (2010)
 Tibolone	T98G cells mRNA ↑ Ngb ↑	Menopause hormone therapy	Avila-Rodriguez et al. (2016)
Thyroid hormone			
 Triiodothyronine	Male rats' hippocampus, cerebellum and cortex mRNA ↑ Ngb ↑	Early stages of hypothyroidism	Oliveira et al. (2015)
Plants derivatives			
Polyphenolics compounds			

 Naringenin	SK-N-BE mRNA n.d. Ngb ↑	MCF-7 mRNA n.d. Ngb no change	n.d.	De Marinis et al. (2010); Cipolletti et al. (2019)
 Biochanin A	N2a, SKNSH mRNA ↑ Ngb ↑		n.d.	Liu et al. (2016)
 Genistein	N2a, SKNSH mRNA ↑ Ngb ↑	MCF-7 mRNA n.d. Ngb no change	n.d.	Liu et al. (2016); Cipolletti et al. (2019)
 Polydatin	N2a, SKNSH mRNA ↑ Ngb ↑		n.d.	Liu et al. (2016)
 Formononetin	N2a, SKNSH mRNA ↑ Ngb ↑		n.d.	Liu et al. (2016)
 Daidzein	N2a, SKNSH mRNA ↑ Ngb ↑	MCF-7, T47D mRNA n.d. Ngb ↓	n.d.	Liu et al. (2016); Montalesi et al. (2020)
 Equol		MCF-7, T47D mRNA n.d. Ngb ↑	n.d.	Montalesi et al. (2020)
 o-desmethylangolensin		MCF-7, cells mRNA n.d. 0,1 μmol Ngb ↑,	n.d.	Montalesi et al. (2020)

		1 μ mol no change		
 Daidzein 4-Sulfate		MCF-7, T47D	mRNA n.d. Ngb \downarrow	n.d. (Montalesi et al., 2020)
 Daidzein 7-Sulfate		MCF-7	mRNA n.d. Ngb \uparrow	n.d. Montalesi et al. (2020)
 Daidzein 4,7-disulfate		MCF-7	mRNA n.d. 0,1-1 μ mol Ngb \uparrow 1 μ mol no change	n.d. Montalesi et al. (2020)
 8-Prenylnaringenin		MCF-7	mRNA n.d. Ngb \uparrow	n.d. Cipolletti et al. (2019)
 Quercetin		MCF-7	mRNA n.d. Ngb \uparrow	n.d. Cipolletti et al. (2019)
 Resveratrol	SK-N-BE	MCF-7	mRNA n.d. Ngb \uparrow	n.d. Cipolletti et al. (2019)

Other plants derivatives				
 <p>Miraxanthin-III</p>		Molecular docking mRNA n.d. Ngb n.d.	n.d.	Pangestu et al. (2018)
 <p>Strigol</p>		Molecular docking mRNA n.d. Ngb n.d.	n.d.	Pangestu et al. (2018)
Short-chain fatty acids				
<i>Natural</i>				
 <p>Trans-Cinnamic Acid</p>	HN33 cells mRNA ↑ Ngb ↑		n.d.	Jin et al. (2011)
<i>Synthetic</i>				
 <p>Valproic Acid</p>	HN33 cells mRNA ↑ Ngb ↑		Anticonvulsant drug	Jin et al. (2011)
NSAIDs				
<i>Synthetic</i>				
 <p>Ibuprofen</p>		AD rats mRNA n.d. Ngb ↑	NSAIDs	Zara et al. (2013)

 <p>Ibuprofen-lipoic acid</p>	<p>AD rats</p> <p>mRNA n.d.</p> <p>Ngb ↑</p>	<p>NSAIDs</p> <p>Zara et al. (2013)</p>
Antidiabetic		
<i>Synthetic</i>		
 <p>Metformin</p>	<p>Wistar rats with alcohol-induced neurotoxicity</p> <p>mRNA n.d.</p> <p>Ngb ↑</p>	<p>Antidiabetic drug</p> <p>Bonea et al. (2020)</p>

AD: Alzheimer's disease; n.d.: not detected; Ngb: neuroglobin; NSAIDs: nonsteroidal anti-inflammatory drugs