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Graphical Review

The emerging roles of somatic globins in cardiovascular redox biology and beyond

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ABSTRACT

The vertebrate globins are a group of hemoproteins with the intrinsic capacity to regulate gaseous ligands and redox signaling required for cardiovascular biology. This graphical review will provide a comprehensive synopsis of somatic cardiovascular globins focusing on expression, function and redox signaling – an emerging area in both physiology and disease.

Contents

Introduction

The globins are a family of small globular proteins that structurally consist of a conserved "globin fold" and a series of eight alpha-helical segments (named A-H) [1–3]. Globins evolved from a common ancestor, four of which reside in vertebrates: hemoglobin (Hb), myoglobin (Mb), cytoglobin (Cgb) and neuroglobin (Ngb) [4]. Harboring a heme-prosthetic group (Fe-protoporphyrin IX), Hb and Mb are penta-coordinate, whereas Cgb and Ngb possess a hexa-coordinate configuration. Globins predominantly operate to reversibly bind gaseous ligands, such as oxygen (O_2) and carbon dioxide (CO_2), to the heme group for storage and transport. This property permits globins to fulfill obligatory aerobic respiratory needs for tissue function and homeostasis. In addition to their respiratory function, it has been long known that globins, especially Hb and Mb, take on other cellular roles such as reduction and oxidation (redox) reactions with ligands such as nitric oxide (NO), nitrite (NO_2^-), and hydrogen peroxide (H_2O_2) [5,6]. These functions are critically important in vascular cells, both of

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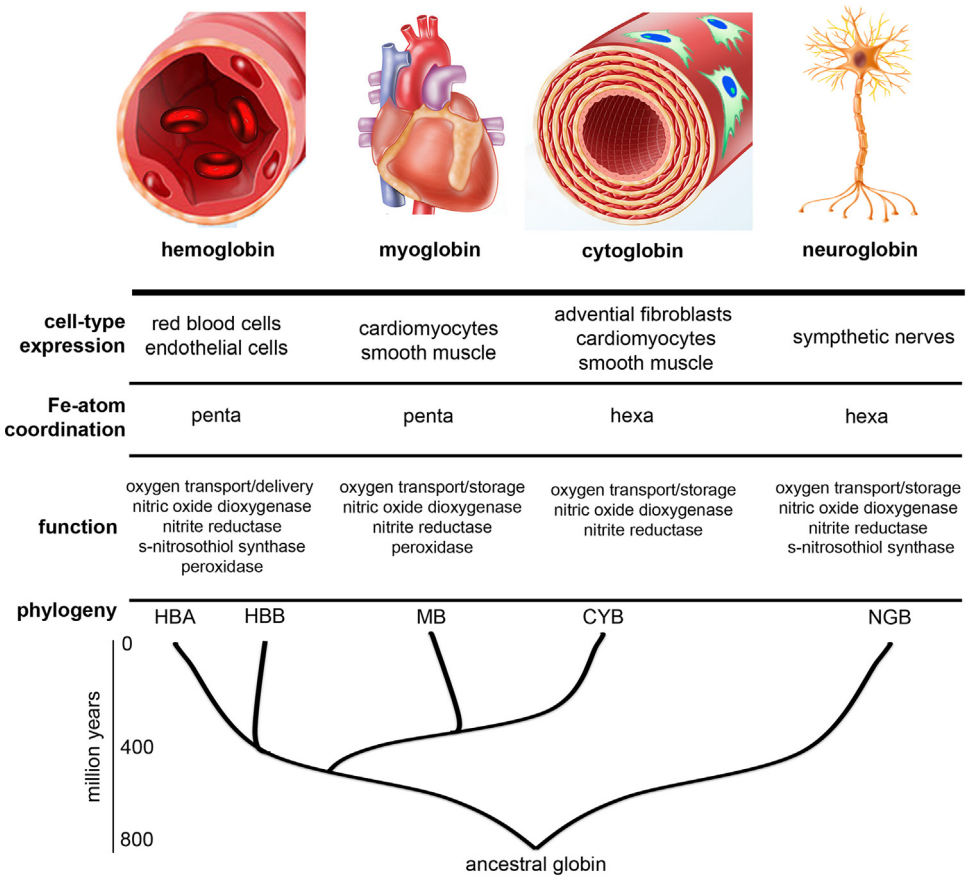

Fig. 1. Schematic outline of the vertebrate globins in the cardiovascular system highlighting cell-type specific expression, biochemical functions and phylogenetic features.

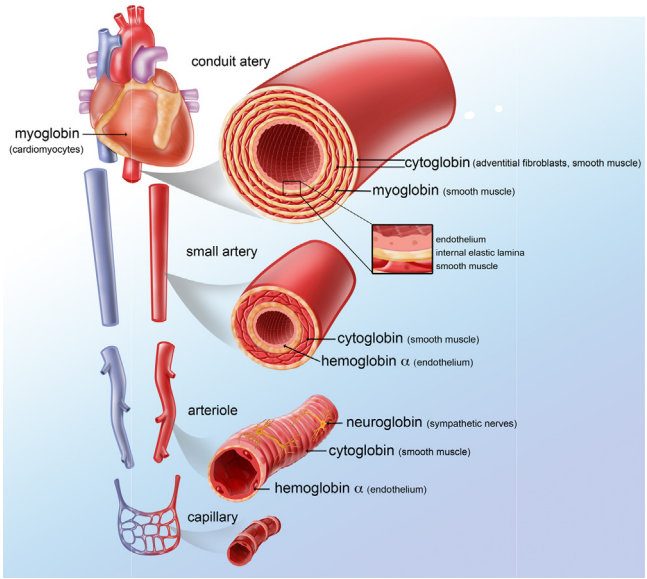

Fig. 2. Cell type-specific expression of hemoglobin, myoglobin, cytoglobin and neuroglobin along the vascular tree.

hematopoietic and of somatic origin. Recently, somatic cardiovascular globin expression and function has received much attention for their roles with regard to redox reactions that control reactive oxygen and nitrogen signaling (Fig. 1).

Within the cardiovascular system, cell-type expression and localization of each globin is variable. Hb, composed of α and β chains, was traditionally known for exclusive expression in red blood cells, although a recent reports have dismissed this perception through the discovery of Hb α expression in microvascular endothelial cells [7,8]. Mb expression is abundant in cardiomyocytes but is found to a lesser extent in vascular smooth muscle cells [9,10]. Cgb chiefly resides in cardiomyocytes, advential fibroblasts and vascular smooth muscle cells [11,12], whereas sympathetic nerves express Ngb [13]. Remarkably, globin expression is not homogeneous through the vascular tree. This is particularly true for Hb and Mb. Hb α expression is mainly found in resistance arteries and arterioles [7], whereas Mb is primarily expressed in conduit arteries but absent in small arteries [7,10] (Fig. 2). The explanation for globin expression inconsistency throughout the vascular tree remains unknown. These disparities likely reflect specific transcriptional, post-transcriptional, and translational events for each globin as it relates to their precise role (s) within the vascular cell itself.

Hemoglobin, myoglobin, cytoglobin and neuroglobin function in the cardiovascular system

Hemoglobin

Hemoglobin A is the major form in adult red blood cells and is encoded by duplicated HBA1 and HBA2 genes and by the HBB gene (reviewed in [14]). Hb protein is assembled into heterotetramers, comprised of two α-globin and two β-globin polypeptides, capable of binding multiple gaseous ligands such as O_2, carbon monoxide (CO), and NO [15]. In the cardiovascular system, red blood cell Hb is best known to mediate O_2 delivery and the removal of CO_2 from peripheral tissues. Hb accomplishes this by cooperatively binding O_2 in the lungs and transporting it to capillaries, where it is deployed to parenchymal cells and exchanged for CO_2 [3,16–18]. Mechanistically, these functions are carried out through the heme group, which contains an iron atom (Fe) in the reduced state

(Fe^{2+}), where five of the six coordination sites of the Fe are occupied, four by the porphyrin ring and one by the proximal histidine (His) of the surrounding globin polypeptide. The sixth coordination site is bound reversibly by O_2 and CO_2. During the cardio-respiratory cycle, delivery and removal of O_2 and CO_2 induces no chemical (covalent) or redox change to the heme Fe group.

Besides oxygen, Hb is well known for its reactivity with NO, a powerful vasodilator [19,20]. In fact, the ability of oxygen-bound Hb (oxy-Hb) to scavenge NO was a defining feature that unveiled NO as endothelium derived relaxing factor [21]. The biochemical reaction of oxy-Hb and NO has largely been ascribed to the dioxygenation reaction, which is fast ($2.4 \times 10^7\ M^{-1}s^{-1}$) and irreversible, resulting in a two-electron oxidation of NO forming met-Hb (Hb-Fe^{3+}) and nitrate (NO_3^-) [22–24]. As a consequence, oxy-Hb severely limits NO diffusion with a half-life of 0.5 μs. Although this provides a mechanism to limit NO toxicity, the reaction creates a physiological paradox, whereby abundant concentrations of Hb in red blood cells (~10 mM) should reduce NO bioavailability from endothelium to vascular smooth muscle. However, in the resistance vasculature – arteries and arterioles that control blood pressure and flow – recent evidence identified that endothelial nitric oxide synthase (eNOS) polarizes and enriches at the myoendothelial junction, the structural location where endothelium and smooth muscle juxtapose [25]. This spatial polarization provides a mechanism that the resistance arterial vessels use to protect eNOS-derived NO from oxy-Hb in red blood cells. Despite this anatomical safeguard from red blood cell Hb, the Hb-NO paradox was further compounded with the recent identification of Hb α expression and enrichment at myoendothelial junctions [7]. This leads to uncertainty, questioning why the resistance arterial endothelium evolved to "sandwich or encapsulate" eNOS with Hb.

One explanation could be extrapolated from a functional point of view. Vascular resistance and blood flow are hypersensitive to arterial radius fluctuations, which can be derived from Poiseulille's law. Poiseulille's law states that "vessel resistance is directly proportional to the length of the vessel and the viscosity of blood and inversely proportional to the radius to the fourth power". For example, a 2-fold increase in radius yields a 16-fold decrease in vascular resistance/blood flow. From an operative point of view, this commands precise and highly controlled mechanisms for regulation of vasodilation and vasoconstriction. NO, which is not directional and diffusion limited, may not offer the precise level of control required for optimally regulating vascular resistance and blood flow. In fact, it is well established that the NO pathway plays a relatively minor role in resistance arterial vasoreactivity. For example, in mesenteric arteries acetylcholine-induced vasodilation is regulated mainly by endothelial derived hyperpolarizing factor (EDHF) [26,27]. This concept also holds true for vasoconstriction. It is known that during contraction, signals originating from vascular smooth muscle disseminate to endothelium to activate vasodilatory pathways, including NO signaling [25,28]. Overproduction of NO is known to inhibit the contractile response [7,25], suggesting that controlled NO diffusion to vascular smooth muscle is crucial for vascular tone regulation. This positions Hb as an ideal protein to control NO bioavailability in the microcirculation during agonist-induced activation. This questions why the NO pathway is active in the microcirculation. Although the classic agonist-induced NO-stimulated cGMP pathway does not play a major role in vasodilation and vasoconstriction, NO signaling is fundamental for regulating other elements of vascular signaling including basal vascular tone, protein S-nitrosation, anti-coagulation, anti-proliferation and anti-inflammatory signaling.

One of the most remarkable functions of Hb α in the resistance arterial endothelium is its capacity to deactivate NO via the Hb oxidation state [7]. During agonist-induced stimulation with either acetylcholine or phenylephrine, it was shown that met-Hb α permits NO signaling to smooth muscle through a slow, weak interaction with heme Fe ($3.3 \times 10^3\ M^{-1}\ s^{-1}$), whereas oxy-Hb α inhibited signaling through the fast, irreversible dioxygenation reaction ($2.4 \times 10^7\ M^{-1}\ s^{-1}$). It was discovered that met-Hb reductase or cytochrome B5 reductase 3 (CytB5R3) was critical for the redox cycling from met-Hb α to oxy-Hb α. This paradigm highlights an emerging, yet critically important role for Hb redox state, whereby NO signaling can be switched "on-or-off" (Fig. 3). This evidence may provide a plausible explanation for understanding the Hb-NO paradox in the physiology of blood pressure and flow control. Intriguingly, this paradigm of Hb-NO regulation in somatic cells may go beyond arterial endothelial cells. In fact, many other cell types including Type II alveolar epithelial cells [29–31], renal mesangial cells [32], hepatocytes [33], macrophages [34], neurons [35–39] and endometrial cells [40] express Hb (Fig. 4).

Historically, Hb has been considered to solely react with NO in a manner to suppress the NO signal through the dioxygenation reaction as described above. This reaction requires O_2 bound Hb. Amazingly, Hb has the capacity to switch from a NO scavenger to a NO synthase under low O_2 levels [41]. In doing so, hypoxic conditions facilitate Hb-to reduce nitrite, resulting in NO formation. Currently, it is known that red blood cell hemoglobin is a nitrite reductase but it is unknown whether Hb α serves as a nitrite reductase in endothelial cells. Another function for Hb is its capability to generate S-nitrosothiols, which are important signaling molecules for post-translational modification of proteins. Hb can synthesize these molecules via reductive nitrosylation [42–44] or through a nitrite anhydrase reaction [45–47]. Collectively, NO and its interaction with Hb assimilates a relationship that is

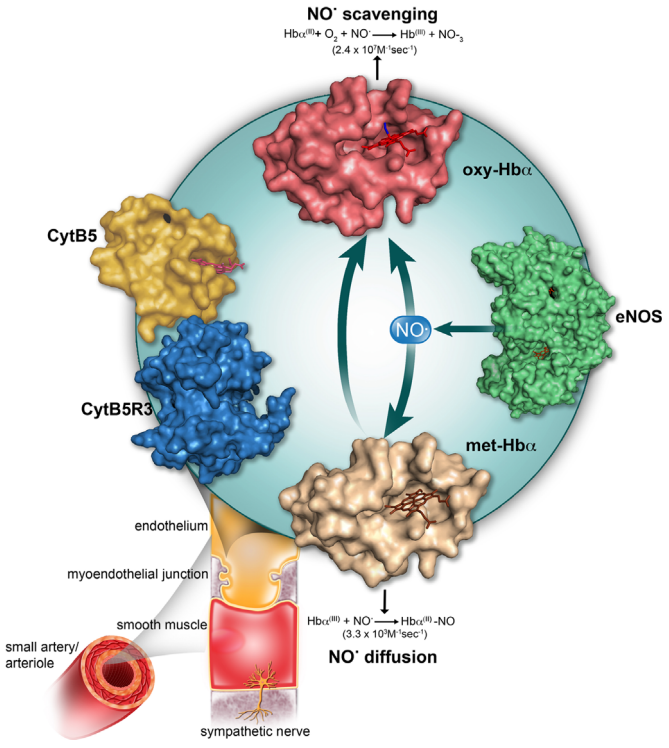

Fig. 3. Mechanism of Hb α-regulated NO signaling in endothelial cells. Hb α is expressed in small artery and arteriolar endothelial cells and enriched at the myoendothelial junction (lower left hand corner). Nitric oxide, released from eNOS, reacts with oxy-Hb $\alpha^{Fe^{2+}}$ resulting in NO scavenging (dioxygenation reaction) or reacts with Hb $\alpha^{Fe^{3+}}$ resulting in a slow and weak binding NO allowing for diffusion. The cycling of Hb $\alpha^{Fe^{3+}}$ to Hb $\alpha^{Fe^{2+}}$ is controlled by a CytB5 and CytB5R3 dependent mechanism.

bound by O_2, whereby the biochemical and signaling nature of NO is highly controlled by O_2 tension.

Hemoglobin also has peroxidative properties when it reacts with oxidants such as hydrogen peroxide (reviewed in [48]). Excessive intravascular Hb exposure can result in profound tissue damage by causing lipid peroxide protein modifications and by scavenging NO [49,50]. However, Hb can also have protective effects, suggesting that the biological consequences of Hb in a H_2O_2-rich environment likely entails a balance of anti-oxidant function and protection or excessive reactive oxygen species and oxidative stress [51]. In fact, recent work demonstrated that Hb expression in renal mesangial cells and hepatocytes attenuate oxidative stress. Thus it is possible that Hb, particularly in endothelial cells, may have other functions besides control of NO signaling as well as possible roles in reactive oxygen signaling and stress responses.

Myoglobin

Mb, a monomeric heme-bound globin, is well known for its ability to store oxygen within cells [9,52]. Like Hb, Mb has the capacity to bind small molecule ligands such as O_2, NO and CO at its sixth coordinate position. In the cardiovascular system, myoglobin protein is abundantly expressed in the cytoplasm of cardiomyocytes and to a much lesser extent vascular smooth muscle [9,53]. Functionally, Mb can fulfill many roles within vascular cells. Initial work found that Mb can reversibly bind O_2 and showed a hyperbolic O_2 dissociation curve. In cardiac muscle, Mb has a high affinity for O_2 with a P_{50} of 1 Torr, with the cytosolic pool of Mb maintained between 35% and 50% oxygen saturation [9]. Based on this biochemical evidence, particularly in working muscle such as cardiomyocytes, Mb is poised as an ideal candidate for short-term O_2 storage and delivery in times of hypoxic challenge. Currently, this function for Mb is the most accepted. To a lesser extent, Mb may mediate facilitated oxygen diffusion in vivo [6].

Similarly to Hb, Mb has NO dioxygenation activity with a reaction rate of $5\times10^7\,M^{-1}\,s^{-1}$ [54,55]. It is thought that the dioxygenase function of Mb prevents NO mediated inhibition of mitochondrial respiration, which would facilitate a higher rate of oxidative phosphorylation [56]. Perhaps one of the most important functions of myoglobin is its ability to serve as a nitrite reductase [57]. Nitrite is found basally at approximately 1–10 μM in the heart and can be reduced to bioactive NO [58]. Under low oxygen conditions, it has been demonstrated that myoglobin inhibits mitochondrial respiration in cardiomyocytes below the P_{50} of myoglobin [57]. Together, these studies highlight the important role of myoglobin regulated NO signaling.

It is critical to point out that Mb also functions as an intracellular catalyst. In vitro studies have demonstrated that Fe^{2+} and Fe^{3+} Mb react with reactive oxygen species particularly H_2O_2, in a similar fashion to Hb [59]. The reaction with H_2O_2 leads to Fe^{4+}–Mb formation, a powerful oxidant that can oxidize proteins and lipids. Fe^{4+}–Mb can react with protein radicals forming cross-linked species as well as initiating lipid peroxidation. This oxidative chemistry may be a detrimental pathway that initiates and exacerbates many cardiovascular related diseases.

Cytoglobin

Cytb, a new member of the globin family displays comparable tertiary structure and the globin fold analogous to Hb and Mb, however, it resides in a dimeric quaternary state [60]. Cytb protein is expressed in varying tissues types [61] compared to the specific expression patterns of Hb and Mb. In the vasculature, Cytb is abundantly found in vascular smooth muscle, adventital fibroblasts and cardiomyocytes, while endothelial cells express little protein [11,12]. Functionally, Cytb is localized to the cytosol, implying that it likely plays a role in O_2 storage similar to Mb [12]. The P_{50} for O_2 is comparable to Mb (~1 Torr), however, the binding of O_2 is cooperative [62]. This indicates that Cytb may permit greater O_2 loading and unloading with a narrow range of O_2 tensions, making it an ideal protein for tissue O_2 storage and deployment during hypoxic challenge. During hypoxia, Cytb mRNA is abundantly expressed in the adult heart, translating into a marked increase of Cygb protein within the hypoxia-induced hypertrophic myocardium. The regulation of Cygb transcription was reported to be calcineurin-dependent suggesting that Cygb may have a functional role in calcium-dependent events accompanying cardiac remodeling [11].

Cygb consumes NO through the dioxygenase pathway, which regulates cell proliferation and respiration [12,62–64]. Recent work demonstrated that O_2-dependent NO metabolism can be controlled by rapid reduction of Cytb by the cellular reductant ascorbate [65]. Expression of Cytb, predominantly in vascular smooth muscle, may likely provide another layer of NO control for vascular tone regulation, especially in the microcirculation. Similar to Hb, cytoglobin can also serve as a nitrite reductase, generating NO and activating soluble guanylyl cyclase in vascular smooth cells [66]. In addition Cytb can also serve as a S-nitrosthiol synthase [67]. Future studies using Cytb knockout mice will provide valuable insight into other potential roles for Cytb in cardiovascular biology [68].

Neuroglobin

The most distantly related globin found in the human genome is Ngb, encoded by NGB [69]. Ngb is related to the invertebrate

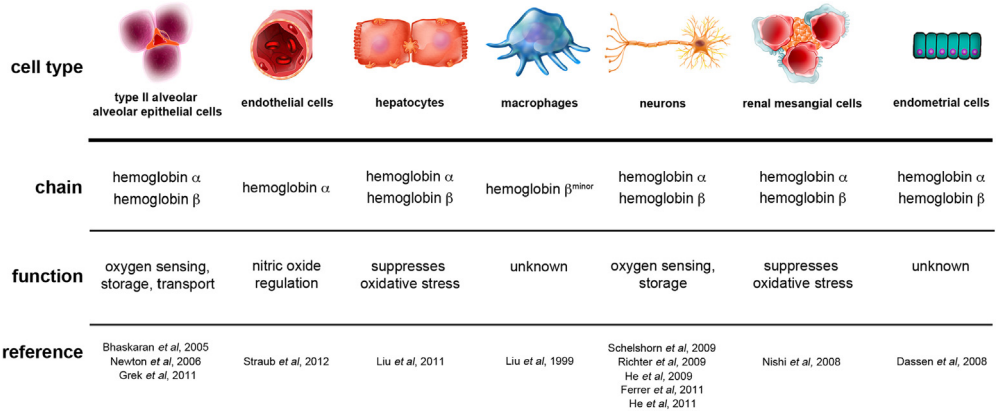

Fig. 4. Known somatic cell types that express hemoglobin, the identified chains, and functions.

nerve globins, indicating that an ancestral gene was present before the divergence of vertebrates and invertebrates more than 800 million years ago [70]. Ngb mRNA is abundant in mammalian nerve cells of the central and peripheral nervous system [13]. In the cardiovascular system, sympathetic nerves express Ngb, however, no expression was found in systemic endothelium or vascular smooth muscle [7,13]. Hypoxia has been shown to increase Ngb expression [71]. The O_2 affinity of Ngb is sensitive to changes in pH and temperature, which indicates that Ngb may act to facilitate O_2 supply [62]. Ngb may also function as a scavenger for reactive oxygen and nitrogen species functioning in a neuroprotective role after ischemia and reperfusion in the brain [72]. However, EPR and kinetic studies revealed that the binding of NO to Ngb-Fe^{2+} is relatively low compared to penta-coordinate Hb and Mb [72]. Ngb can also serve as a nitrite reductase, in which redox sensitive surface thiols are critical for the binding of nitrite and the production of NO [73]. These thiols are likely linked to the redox state of the cell, which may control Ngb function [64]. The role of Ngb in perivascular sympathetic nerves remains unknown, however, it may contribute to catecholamine release or neuronal nitric oxide synthase-derived NO regulation.

Future directions

While the field of cardiovascular globin biology is evolving, several challenges remain. First, the transcriptional, post-transcriptional and protein–protein interactions that regulate each globin in somatic vascular cells are poorly understood and will require more study. Second, further work is necessary to understand how the redox state of each globin is controlled in somatic vascular cells. Lastly, additional effort will be needed to dissect the individual and communal roles of globins and how they function in both cardiovascular physiology and disease.

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