S-nitrosylation: integrator of cardiovascular performance and oxygen delivery

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Delivery of oxygen to tissues is the primary function of the cardiovascular system. NO, a gasotransmitter that signals predominantly through protein S-nitrosylation to form S-nitrosothiols (SNOs) in target proteins, operates coordinately with oxygen in mammalian cellular systems. From this perspective, SNO-based signaling may have evolved as a major transducer of the cellular oxygen-sensing machinery that underlies global cardiovascular function. Here we review mechanisms that regulate S-nitrosylation in the context of its essential role in "systems-level" control of oxygen sensing, delivery, and utilization in the cardiovascular system, and we highlight examples of aberrant S-nitrosylation that may lead to altered oxygen homeostasis in cardiovascular diseases. Thus, through a bird's-eye view of S-nitrosylation in the cardiovascular system, we provide a conceptual framework that may be broadly applicable to the functioning of other cellular systems and physiological processes and that illuminates new therapeutic promise in cardiovascular medicine.

Oxygen and NO: co-evolution for common function

From the appearance of the simplest metazoans to the most complex multicellular life forms, the ability to efficiently handle oxygen has remained essential for survival and has therefore been subject to intense evolutionary pressure. A single-cell organism must rapidly adapt its core homeostatic processes to fluctuations in oxygen tension, functions retained in specialized cells of higher vertebrates (1, 2). In addition to cell-autonomous pathways for oxygen homeostasis, complex multicellular organisms have also developed sophisticated mechanisms to efficiently coordinate oxygen delivery and utilization across diverse organ systems (3–5). From this perspective, the human cardiovascular system in all its complexity has evolved for the principal purpose of oxygen delivery.

Although oxygen itself can function as a signaling molecule (2, 6), its signaling repertoire is dependent largely on heme binding and is therefore limited, as hemes do not generally convey cellular signals. Thus, organisms have necessarily evolved parallel mechanisms to precisely control oxygen flux and function. Utilization of the ancient gasotransmitter NO, highly abundant in the primordial atmosphere and linked to anaerobic respiration, likely co-evolved with oxygen to serve a common function — regulation of aerobic respiration (i.e., oxygen delivery and utilization). While NO, like O_2, binds transition metal centers to elicit cellular signals, the majority of its cellular influence is achieved through posttranslational modification (PTM) of cysteine thiols, a process termed S-nitrosylation (7). The universal presence of cysteine thiols in all major classes of proteins greatly expands signaling possibilities, and regulation of protein function via S-nitrosylation may be viewed as the prototypical system for redox-based and gasotransmitter-mediated signal transduction (8).

Recent reviews of S-nitrosylation have detailed the redox biochemistry of reactive nitrogen species (8–10) and cataloged the myriad proteins and cellular processes known to be regulated by this modification across systems, including the cardiovascular system (11, 12). Nearly 1,000 S-nitrosylated proteins have been identified in the

heart alone (13, 14), and cross-talk with a plethora of other PTMs has been described (15). Principles underlying reversibility, specificity, and enzymatic control of S-nitrosylation have received particular attention. Here, we take a thematic perspective that highlights the essential role of protein S-nitrosylation in the systems-level control of oxygen delivery and utilization, which is arguably the essential function of the cardiovascular system. Using these physiological insights, we highlight examples of how S-nitrosylation is dysregulated in cardiovascular disease and how modulation of this signaling mechanism holds therapeutic promise. Through this bird's-eye view of S-nitrosylation in the cardiovascular system, we provide a conceptual framework that may be broadly applicable to cellular systems, physiological processes, and diseases.

S-nitrosylation as a prototypical system of protein PTM
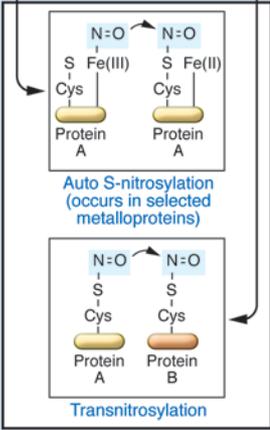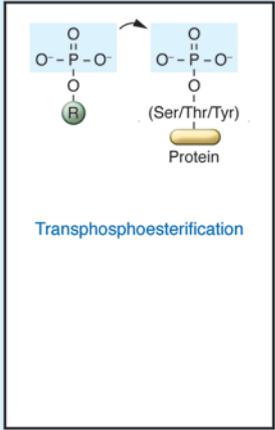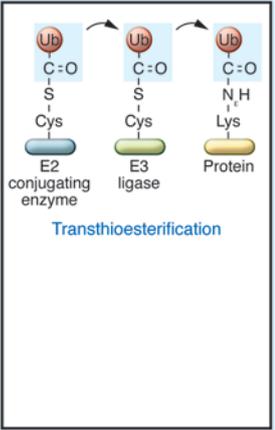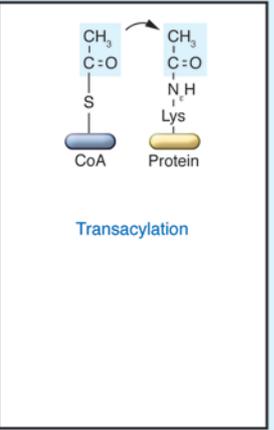
Systems governing PTM of proteins generally fall into two broad categories, those with a ubiquitous sphere of influence (e.g., phosphorylation) and those with a more limited cellular purview (e.g., methylation). S-nitrosylation, like phosphorylation, is clearly evolutionarily conserved and ubiquitous, affecting most, if not all classes of proteins across all cellular compartments (8, 9, 16). By contrast, other oxygen/redox-based modifications, including hydroxylation and sulfenylation, have been identified to date with specific classes of proteins and functions (2, 17). Here, we draw parallels between S-nitrosylation and other important PTMs (e.g., phosphorylation, ubiquitinylation, acetylation) to provide a conceptual framework for understanding the molecular machinery that governs this fundamental biologic process (Figure 1).

In mammals, the principal sources of newly synthesized NO are the three NOS isoforms (NOS1–3). Nitrate and nitrite may also contribute to the NO reservoir (18, 19), particularly under duress. The transfer of the NO moiety to cysteine thiols in target proteins is carried out by peptide or protein nitrosylases, which mediate either metal-to-Cys or Cys-to-Cys transfer. Metal-to-Cys nitrosylases are proteins that transfer NO groups from transition metals (e.g., Fe^{2+}, Cu^{2+}) to cysteine thiol. For example, mammalian hemoglobin (Hb) undergoes auto-nitrosylation via intramolecular transfer of NO from heme iron (iron nitrosyl; HbFeNO) to a

A

	S-nitrosylation	Phosphorylation	Ubiquitinylation	Acetylation
Moiety	−N=O NO group	O=P with O⁻ groups	Ubiquitin polypeptide (N—□—C)	−C(=O)−CH₃
Source	NOSs (NOS1/2/3)	ATP	Ubiquitin	Acetyl-CoA
Ubiquitous?	Yes Occurs across all functional classes of proteins and cellular compartments	Yes Occurs across all functional classes of proteins and cellular compartments	Yes But with a predominance for protein degradatory pathways	Yes But with a dominant role in transcription and metabolic signaling
Enzymatic machinery mediating transfer	Nitrosylases Metal → Cys Cys → Cys	Protein kinases (Ser/Thr/Tyr)	E1 – ubiquitin-activating enzyme E2 – ubiquitin-conjugating enzyme E3 – ubiquitin ligase	Acetyltransferase
Transfer biochemistry	Auto S-nitrosylation (occurs in selected metalloproteins) Transnitrosylation	Transphosphoesterification	Transthioesterification	Transacylation
Enzymatic machinery mediating removal	Denitrosylases (see panel **B**)	Protein phosphatases	Deubiquitinating enzymes	Deacetylases
Specificity determinants	Spatiotemporally confined interactions between substrates and sources Enzymatic machinery Motifs (alignment of enzymes and specific substrate sites and regulation of thiol pK) Can be stimulus coupled	Essentially the same as for S-nitrosylation	Essentially the same as for S-nitrosylation	Essentially the same as for S-nitrosylation

B

S-nitrosoglutathione reductases (GSNOR) systems

Thioredoxin systems
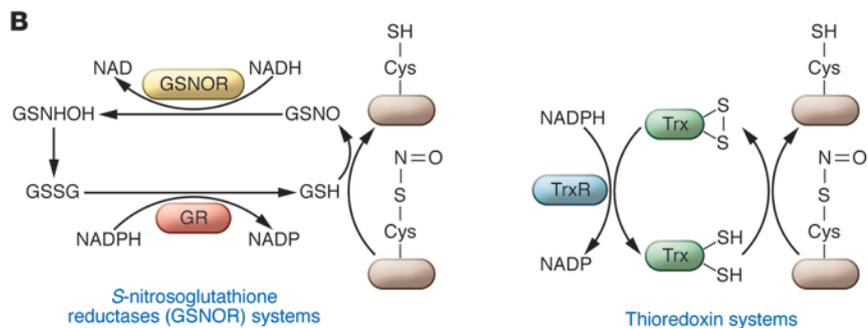
Figure 1

Framework for PTMs and signaling. (**A**) Parallels between *S*-nitrosylation and other PTMs (phosphorylation, ubiquitinylation, and acetylation) highlight shared features of bona fide signaling systems. *S*-nitrosylation is ubiquitous, reversible, and subject to enzymatic control (by nitrosylases and denitrosylases), enabling spatiotemporal and target specificity. The biochemistry of NO group transfer and denitrosylation reactions is depicted. Note that auto–*S*-nitrosylation is shown as an example of metal-to-Cys NO transfer (e.g., as occurs from the Hb heme center to Cysβ93 to form SNO-Hb). However, metal-to-Cys NO transfer between two different peptides or proteins may also occur (e.g., between cytochrome C and glutathione to form GSNO). (**B**) Denitrosylases. Two classes of denitrosylases are shown, which comprise four enzymes in mammals, including two GSNORs and two thioredoxins. GR, glutathione reductase; GSH, reduced glutathione; GSNHOH, glutathione *N*-hydroxysulfenamide; GSSG, oxidized glutathione; TrxR, Trx reductase.

specific cysteine thiol in the β-globin chain (Cysβ93) (20, 21). Similarly, transfer of metal-coordinated NO from cytochrome *c* (22) or ceruloplasmin (23) is employed in the synthesis of *S*-nitrosoglutathione (GSNO). Cys-to-Cys nitrosylases (referred to herein as transnitrosylases) are typically *S*-nitroso-proteins (SNO-proteins) involved in Cys-to-Cys transfer of the NO group from donor to acceptor protein (9, 24). The net nitrosylation (NO group transfer) reaction is analogous to the group transfer reactions catalyzed by ubiquitin ligases, palmitoyltransferases, or acetyltransferases (Figure 1). Conversely, protein denitrosylation has been shown to play a major role in decreasing cellular *S*-nitrosylation (25–29) much the same way that protein phosphatases, deubiquitinases, and deacetylases set cellular levels of their respective PTMs (9, 11). To date, two major enzymatic systems mediating protein denitrosylation have been described (Figure 1): GSNO reductase (GSNOR) (30, 31) and thioredoxin (Trx) (26, 32). The GSNOR system (two GSNORs have been identified, but only one has been studied in detail; ref. 25) denitrosylates GSNO, the major low-molecular-weight SNO in mammalian cells (33). Although GSNOR acts directly only on GSNO, it governs protein *S*-nitrosylation by influencing the cellular equilibrium that is maintained by transnitrosylation reactions between SNO-proteins and GSNO (8, 24). Studies of GSNOR-deficient organisms have demonstrated the central role of GSNOR in SNO-based signal transduction and cardiovascular function (refs. 27–29, 34, 35, and Figure 2). Members of the TRX enzyme family (TRX1 and TRX2) mediate the denitrosylation of multiple SNO-protein substrates in the cytosol and mitochondria (25, 36).

Studies over the past decade have established close parallels between guiding principles for *S*-nitrosylation (SNO-based signaling) and other PTM systems. Signals are propagated through stimulus-coupled and spatiotemporally restricted interactions within signaling complexes. The composition of SNO-based signaling complexes includes NOSs (which provide the source of NO), NO group donors including GSNO and other SNO-proteins that can participate in transnitrosylation (propagation) reactions, and denitrosylases (which curtail the signals) (Figure 1). By analogy to kinases, which align with substrates through hydrophobic and ionic interactions, hydrophobic and charged amino acids surrounding substrate cysteines ("SNO motifs") may provide for alignment with nitrosylases (8, 9, 37). Thus, SNO-protein abundance reflects regulated equilibria between *S*-nitrosylation and denitrosylation pathways, rather than rates

of NO production per se. The next section, which details the role of SNO-based signaling in cardiovascular function and oxygen homeostasis, illustrates the operation of these principles under physiologic and pathophysiologic states.

SNO-based signaling regulates cardiovascular performance and optimizes oxygen delivery

Systemic oxygen delivery is largely determined by microcirculatory blood flow and, to a lesser extent, by blood O₂ content, which is a function of Hb O₂ saturation (SaO₂) and blood Hb concentration. SNO-based signals regulate each of these determinants and therefore play an essential role in optimizing oxygen delivery. Furthermore, *S*-nitrosylation allows for crosstalk between NO and O₂-sensing pathways to signal tissue oxygen levels and to effect changes in O₂ bioavailability (5). Here, we illustrate how the SNO-based system exerts coordinated effects across multiple organs to provide an integrated mechanism for sensing oxygen levels and executing molecular responses to hypoxic cues (Figure 3). Inasmuch as oxygen sensing and delivery are perturbed in all cardiovascular disease, it follows that dysregulated SNO signaling contributes to disease pathogenesis.

S-nitrosylation and myocardial performance during simulated hypoxia. Signaling via the β₂-adrenergic receptor (β₂-AR) coordinates hypoxic adaptation across multiple organs, including the lungs (improved ventilation/perfusion matching) (38–41), skeletal muscle (hypoxic vasodilation [HVD]) (41–43), and heart (augmenting contractility) (44). Through its influence on β₂-AR signaling, *S*-nitrosylation may regulate hypoxic responses. The G protein receptor kinase 2 (GRK2), which mediates β₂-AR desensitization, undergoes agonist-coupled inhibitory *S*-nitrosylation in an eNOS-dependent manner (28). Absent *S*-nitrosylation, cardiac contractility (28) and peripheral vasodilation (45) decline during maintained adrenergic stimulation (28). β-Arrestin2 (a scaffolding protein that targets the β₂-AR for internalization via endocytosis) and dynamin (a core component of the clathrin-mediated endocytotic machinery) also undergo *S*-nitrosylation downstream of the β₂-AR, leading to enhanced receptor trafficking (34, 46). Although the precise chain of molecular events is not fully understood, it is known that GRK2, β-arrestin2, and dynamin are each complexed with eNOS, and stimulation of the β₂-AR leads to eNOS activation and subsequent *S*-nitrosylation of these proteins (34, 46, 47). Thus, coordinate *S*-nitrosylation events may serve to enable β₂-AR signaling by preventing desensitization and promoting receptor recycling to facilitate oxygen transport (cardiac output) and delivery (vasodilation). The abundance of SNO-GRK2 and SNO-β-arrestin2 is diminished in *eNOS*⁻/⁻ mice and enhanced in *GSNOR*⁻/⁻ mice (28, 34); eNOS and GSNOR thus promote *S*-nitrosylation and denitrosylation of these proteins, respectively, through the intermediacy of GSNO. eNOS also binds dynamin (46), but whether *S*-nitrosylation is mediated directly by a transnitrosylase activity of NOS (48, 49) or via GSNO is not known. *GSNOR*⁻/⁻ mice further exhibit increases in cardiac output under basal conditions, reflecting marked peripheral vasodilation (29) as well perhaps as pronounced myocardial angiogenesis that results from stimulatory *S*-nitrosylation of HIF-1α under normoxic conditions (35). In addition, GSNO has direct inotropic effects (50). *GSNOR*⁻/⁻ mice also show constitutive increases in β₂-AR abundance (28), as is seen in ischemia. Collectively, then, enhanced *S*-nitrosylation in *GSNOR*⁻/⁻ mice underlies hypoxia-mimetic changes throughout much of the cardiovascular system (Figure 2). Sim-
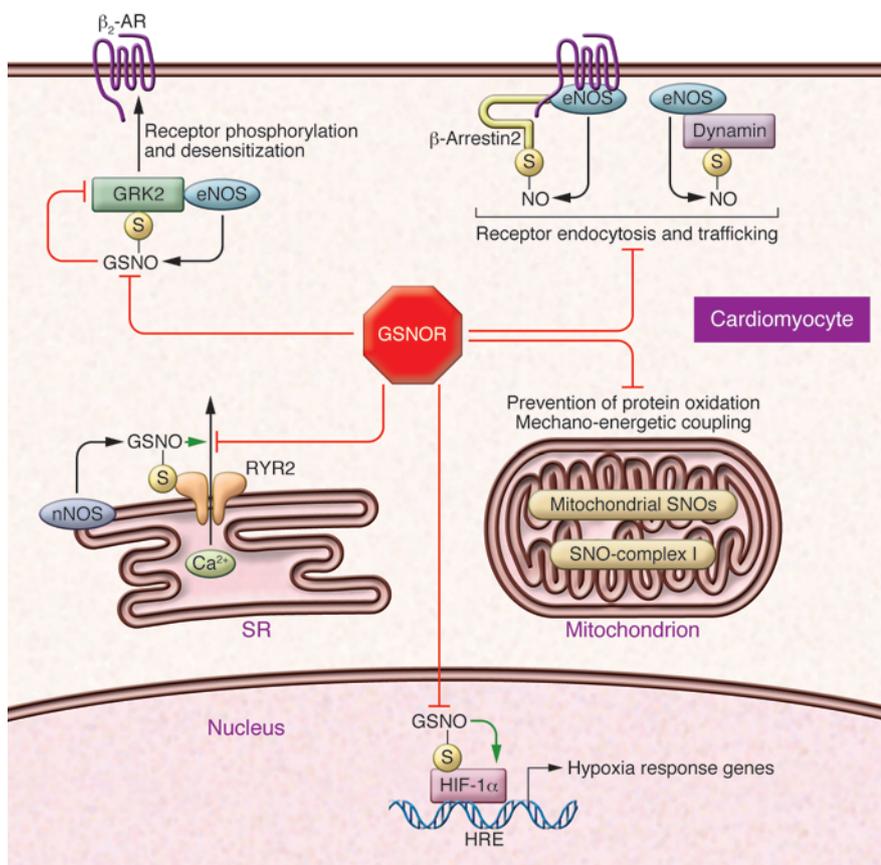
Figure 2
S-nitrosylation regulates cardiomyocyte signaling at critical oxygen-responsive nodal points. The central roles are highlighted for the denitrosylase GSNOR in physiologic control of β₂-AR signaling, SR calcium release, HIF-1α responses, and mitochondrial function. *S*-nitrosylation reactions that have been proven by genetic criteria to occur through the intermediacy of GSNO include those targeting GRK2, RYR2, and HIF-1α. Ligand-dependent *S*-nitrosylation of GRK2, β-arrestin2, and dynamin is eNOS dependent. eNOS is complexed with GRK2, β-arrestin2, and dynamin, as depicted. Coordinate titration of *S*-nitrosylation (envisioned via receptor-coupled activity of transnitrosylases and denitrosylases) across multiple steps in these pathways determine net signaling responses. The effects of GSNOR (from observations in *GSNOR*⁻/⁻ mice) manifest as increases in cardiac output under basal conditions, a persistent state of systemic vasodilation, and protection from ischemic insult, establishing a central role for GSNO in cardiovascular hemodynamics and oxygen delivery. Effects of GSNOR on mitochondrial targets are inferred from studies using GSNO. HRE, hypoxia response element.

ilar SNO-based regulation of β₂-AR signaling in particular, and hypoxia-mimetic responses more generally, are likely operative in other tissues, including the airways and alveoli, kidney, blood, and skeletal muscle, as discussed below (Figure 3).

S-nitrosylation regulates striated muscle performance. Oxygen consumption can increase 5- to 10-fold in exercising humans over the course of minutes (51). Accordingly, skeletal muscle has evolved efficient mechanisms to rapidly adapt to large shifts in oxygen demand. Just as β₂-AR activation increases cardiac contractility during hypoxia (44), β₂-AR–coupled increases in bioactive NO are also critical for compensatory vasodilation during mild to moderate hypoxic exercise (52, 53). In a manner that parallels myocardial β₂-AR signaling, SNO-based signals inhibit β₂-AR receptor desensitization in the periphery to facilitate adrenergic responses (45). Specifically, as local O₂ tension (pO₂) begins to fall during exercise, NO signals to increase blood flow by potentiating β₂-AR–coupled HVD in working muscle (53) via a mechanism that likely involves SNO-GRK2 and inhibition of β₂-AR desensitization (28, 45). As exercise intensity and tissue hypoxia increase, the source of bioactive NO becomes less dependent upon β-adrenergic mechanisms (53) and shifts to rbc-based SNO delivery (3, 4, 10, 54) (see *SNO signaling and the respiratory cycle* below). Together, these two mechanisms may support oxygen delivery across a broad range of exercise intensity and duration.

A parallel mechanism operates in skeletal muscle via hypoxia-dependent, stimulatory *S*-nitrosylation of the skeletal muscle ryanodine receptor (RYR1) (55–57), a key mediator of sarcoplasmic reticulum (SR) calcium release and excitation-contraction

coupling (ref. 58 and Figure 2). *S*-nitrosylation of RYR1 occurs only during hypoxia, increases the open probability (P₀) of the channel, and potentiates SR calcium release (57, 59–62). This pO₂-dependent SNO-RYR1 formation is mediated by NO derived from neuronal NOS (nNOS) complexed with RYR1 (63). In normoxia, by contrast, RYR1 undergoes stimulatory oxidation of redox-sensitive cysteine thiols (55, 60). The source of oxidizing equivalents is the SR-resident NADPH oxidase 4, which colocalizes with RYR1 and produces H₂O₂ in proportion to ambient pO₂, thus functioning as a physiological oxygen sensor (55). Accordingly, both *S*-oxidation and *S*-nitrosylation stimulate RYR1, but at different physiologic pO₂, reflecting conditions from resting to exercising muscle (56). Alternatively stated, when pO₂ falls into the hypoxic range (as occurs in exercising muscle), regulatory thiols in RYR1 become reduced and protein conformation is allosterically altered in a manner that favors *S*-nitrosylation (57). Conversely, *S*-nitrosylation is superseded by *S*-oxidation in the normoxic conformation assumed by RYR1 in resting muscle. Thus, coordinate *S*-nitrosylation and oxidation of Cys thiols within RYR1, which are favored during hypoxia and normoxia, respectively, allow redox control over the range of physiological pO₂. From a pathophysiologic perspective, excessive *S*-nitrosylation of RYR1, which can occur in settings of nitrosative stress, causes SR calcium leak and plays a maladaptive role in Duchenne muscular dystrophy (64), malignant hyperthermia (65), and exercise intolerance (66). Inasmuch as skeletal muscle dysfunction is commonly present in chronic heart failure (67, 68), perturbations in SNO-based signaling may underlie pathological

crosstalk between these two tissues. Targeting key SNO-proteins common to both tissues (e.g., HDAC2) (69–72) may represent a new therapeutic approach.
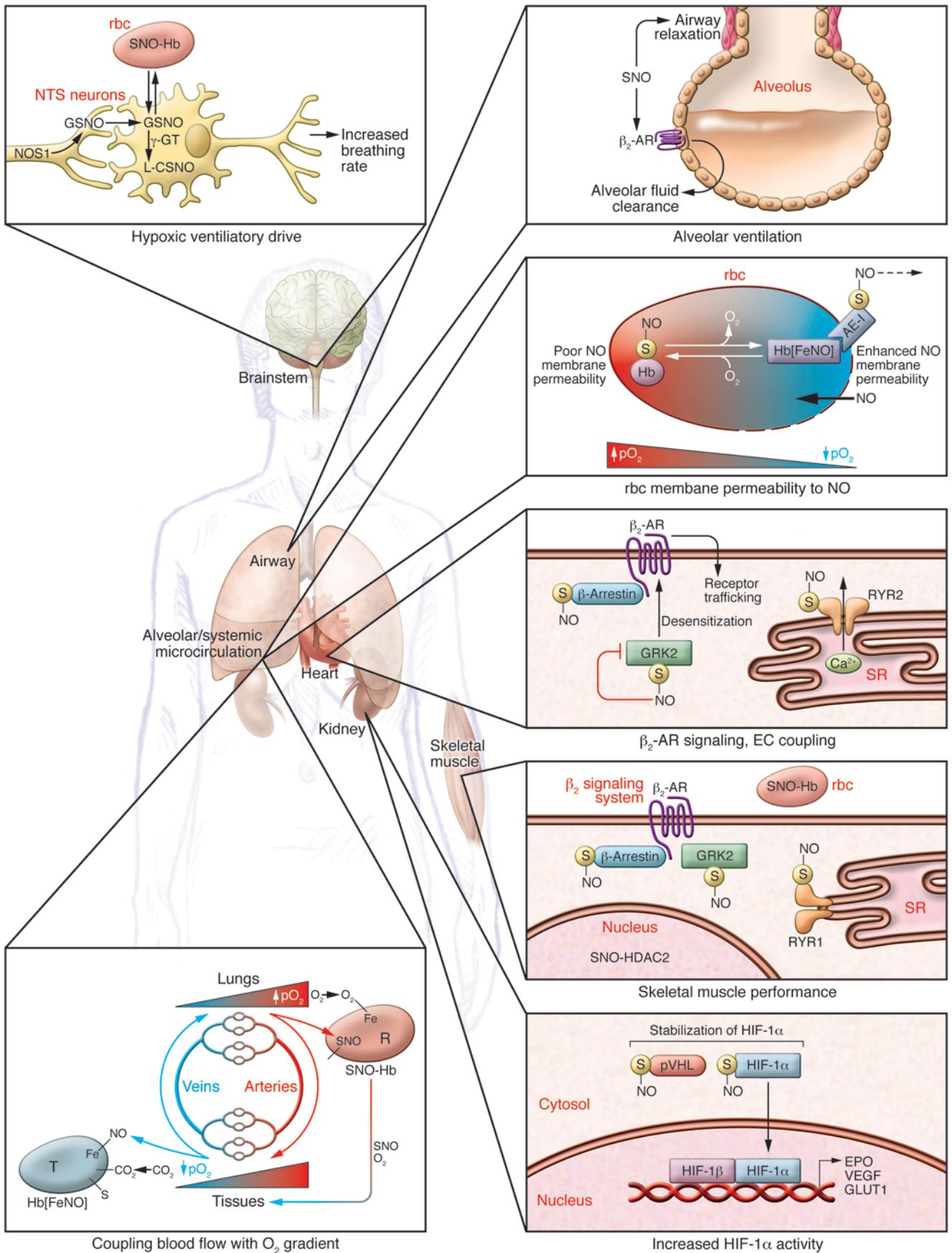
Like skeletal muscle RYR1, RYR2 in cardiac muscle also undergoes nNOS-dependent stimulatory *S*-nitrosylation (refs. 60, 72, and Figure 2). However, unlike skeletal muscle (where NO can directly modify RyR1), the transfer of an NO group from nNOS to RyR2 requires GSNO (i.e., transnitrosylation) (62). In addition, SNO-RyR2 is abundant during normoxia and stimulates channel activity independently of oxygen concentration (29, 62). However, pO_2 may retain an influence on RYR2 *S*-nitrosylation in the heart through the β_2-AR (see above; specifically, through β_2-AR–coupled RYR2 denitrosylation involving GSNOR) (29). The importance of this regulatory pathway has been established by study of $GSNOR^{-/-}$ mice, which exhibit depressed β-adrenergic inotropic responses, impaired β-agonist–induced denitrosylation of RYR2, and pathological calcium leak (29). Similarly, $nNOS^{-/-}$ hearts have diminished SNO-RyR2, excessive diastolic SR calcium leak, contractile dysfunction, and susceptibility to arrhythmias (73, 74). We note that while the β_2-AR system and RYR2 serve as important examples of SNO-based regulation, *S*-nitrosylation likely controls other aspects of cardiac homeostasis in an oxygen-dependent manner. For example, emergent evidence suggests *S*-nitrosylation of mitochondrial proteins may protect against myocardial ischemia (75), potentially via prevention of pathologic protein oxidation and inhibition of apoptosis. Likewise, inhibitory *S*-nitrosylation of mitochondrial complex I, in certain contexts, may play an adaptive role in mechanoenergetic coupling (ref. 76 and Figure 2). As disruption of the SNO/redox balance in myocytes is a hallmark of human heart failure (77), restoration of this equilibrium may provide a fruitful approach to restoring cardiac performance.

SNO signaling and the respiratory cycle. Oxygen delivery is a function of blood O_2 content and blood flow. The ability to augment blood O_2 content is markedly constrained, varying linearly with Hb concentration and SaO_2. Conversely, modulation of regional blood flow, which is proportional to vessel radius to the fourth power, has a dynamic range encompassing several orders of magnitude. Thus, volume and distribution of local blood flow are the principal determinants of tissue oxygen delivery (10). Mammals have a robust capacity to autoregulate systemic blood flow to dynamically couple local oxygen demand with oxygen delivery — a process termed HVD. The central role of rbc in HVD was established half a century ago by Guyton (78), who showed that HVD is inversely proportional to SaO_2 and recapitulated by rbc containing desaturated but not saturated Hb (78). By contrast, HVD is independent of arterial pO_2 (79–81). Guyton proposed that erythrocytes sequestered a vasoconstrictor in the lungs (78), and the critical importance of SaO_2 (as distinguished from pO_2) was overlooked at the time. Later, rbc were appreciated to liberate vasodilator SNOs during hypoxia. Specifically, circulating rbc transport bioactive NO to the peripheral microcirculation and release it in proportion to locally declining oxygen gradients, in a process governed by changes in the quaternary conformation of Hb associated with changes in O_2 concentration (3, 4, 10, 54, 81). The molecular basis for this effect involves a critical cysteine within the Hb β-chain (Cysβ93) that exhibits dynamic *S*-nitrosylation coupled to Hb allostery (3, 4, 20, 81). Oxygen binding to the heme-iron of Hb promotes a transition from T state (in deoxygenated blood) to R state (in oxygenated blood), during which heme-bound NO is transferred to the thiol group of Cysβ93. This auto–*S*-nitrosylated

cysteine remains hydrophobically buried in the R configuration and thus devoid of vasodilatory activity. With the transition from R to T state as erythrocytes travel to increasingly hypoxic regions of the systemic microcirculation, the NO group on Cysβ93 is exposed to solvent and is released via transnitrosylative transfer to glutathione or thiols of the rbc membrane protein AE-1 to form GSNO and SNO AE-1 (10, 54, 82). In this manner, oxygen itself serves as a principal allosteric regulator that couples physiological release of O_2 and bioactive NO. Inasmuch as blood flow is the principal determinant of O_2 delivery, this remarkable function of Hb represents an elegant means of dynamically matching vasomotor tone with local oxygen gradients (refs. 5, 80, and Figure 2). The physiologic importance of SNO-Hb in human hypoxic adaptation was recently demonstrated in an observational study of healthy subjects undergoing progressive high-altitude acclimatization in the Himalayas (83). Blood concentrations of SNO-Hb progressively increased with ascent and were independently correlated with exercise capacity at high altitude.

SNO signaling and pulmonary gas exchange. NO bioactivity exerts control over ventilation-perfusion (V/Q) matching through a dual mechanism: (a) a permissive action on the β_2-AR (28, 34) (see above), which may improve V/Q matching by enhancing alveolar clearance of fluid (84) and (b) the process of hypoxic pulmonary vasoconstriction (HPV), whereby the pulmonary arterial microcirculation preferentially perfuses well-ventilated alveolar units (refs. 5, 10, and Figure 3). Physiological trapping of NO by erythrocytes involves capture or inactivation of NO by hemes of Hb and serves as an important contributor to HPV (85–88). NO trapping during hypoxia may be facilitated by regulation of rbc membrane NO permeability via conformation-dependent binding of Hb to the rbc transmembrane protein AE-1 (54). In normoxia, the rbc plasma membrane constitutes a significant barrier to NO entry mediated by tight association between the submembrane cytoskeleton and the cytoplasmic domain of AE-1. In hypoxia, Hb binds AE-1 (binding is favored in the T state) and alters the submembrane cytoskeletal scaffold in a manner that increases NO permeability, thereby facilitating NO trapping (54, 89–91). As basal vasodilatory tone in the pulmonary arterial circulation is set by a relatively high level of local NO production (from eNOS), NO trapping during hypoxia provides an important braking mechanism on vasodilation and, consequently, regional pulmonary blood flow. In other words, avid NO trapping by less-well-oxygenated erythrocytes perfusing less-well-ventilated lung units, and attenuation of NO trapping by well-oxygenated erythrocytes perfusing well-ventilated lung units, can facilitate V/Q matching (5). pO_2-regulated NO permeability may also facilitate unloading of bioactive NO from SNO-Hb in the transition from R state to T state in the systemic microcirculation to mediate HVD and in the lungs to mitigate excessive pulmonary vasoconstriction (refs. 10, 53, and Figure 3).

Defects in NO processing by rbc are associated with multiple cardiovascular diseases, including sepsis (excess levels of SNOs in rbc) and pulmonary arterial hypertension (PAH) (decreased rbc SNO levels). In sepsis, uncontrolled production of SNOs (27, 82, 92), known as nitrosative stress, is believed to contribute to multiorgan failure with resultant disruption of NO-based vascular autoregulation, particularly V/Q matching in the lung and shunting in tissues (27, 82, 93). SNO content is increased 20-fold in rbc from humans with septic shock and acute respiratory distress syndrome (27, 82), and vasoactivity of these rbc is dysregulated in a murine lung bioassay (82, 93). The link between pO_2 and SNO delivery

Hypoxic ventiliatory drive

Alveolar ventilation

rbc membane permeability to NO

β_2-AR signaling, EC coupling

Skeletal muscle performance

Coupling blood flow with O_2 gradient

Increased HIF-1α activity

Figure 3
SNO-based integration of oxygen utilization and homeostasis across organ systems. SNO-based signals exert coordinated effects across multiple organ systems to provide an integrated mechanism for sensing oxygen levels and executing molecular responses to hypoxic cues. The roles of SNOs in cardiac and skeletal muscle performance; respiratory cycle functions (vasodilator and vasoconstrictor function of Hb), including HVD and alveolar ventilation and perfusion matching (rbc NO permeability and trapping); the central ventilatory drive; and chronic adaptation to hypoxemia and anemia (HIF-1α signaling) are depicted. SNO-mediated activation of HIF-1α has been demonstrated in multiple tissues, including the kidney (127). Specific details shown in the kidney inset are derived, in part, from observations in the heart, brain, and other tissues. EPO, erythropoietin.

that underlies HVD is also overwhelmed in sepsis (94, 95), possibly due to promiscuous transnitrosylation of exofacial rbc membrane proteins that results in pO$_2$-independent vasodilation. Accumulation of rbc SNOs and loss of allosteric control of SNO release (93, 95) may help explain the severely dysregulated blood flow pattern, which is greatly enhanced but chaotic, in the septic microcirculation. This mechanism is supported by studies of GSNOR$^{-/-}$ mice, which exhibit increased rbc SNO content, decreased basal vascular tone (29), and excessive mortality during experimental models of sepsis (27). Conversely, rbc in patients with PAH and hypoxemia have reduced levels of SNO-Hb but preserved rbc NO trapping, which reduces microcirculatory NO bioavailability. This defect may promote excessive pulmonary vasoconstriction in well-ventilated alveolar units and impair blood flow to hypoxic tissues in the systemic circulation (96). As aberrant vascular autoregulation in both the pulmonary and systemic circulation are hallmarks of advanced heart failure (97), defects in SNO processing by rbc in heart failure (98) likely also contribute to disease progression.

SNOs regulate ventilation. During hypoxia, mammals increase total lung ventilation by augmenting breathing rate (hypoxic ventilatory drive) and tidal volume, both of which are regulated by SNO-based signals. The central limb in this response is classically initiated by hypoxia-sensing cells in the carotid body, which relay to nNOS-rich neurons in the brainstem nucleus tractus solitarius (nTS). nNOS activation in the nTS is critical for the hypoxic ventilatory response and likely involves formation of low-molecular-weight SNOs (99, 100). Injection of low-molecular-weight SNOs, in particular GSNO or S-nitroso-L-cysteine, into rodent nTS dramatically increases minute ventilation in a manner that closely mimics the physiological effects of hypoxia (101). Furthermore, nTS injection of a low-mass fraction derived from deoxygenated blood, which contains Hb-derived SNOs, reproduces the effects of GSNO, whereas a low-mass fraction derived from oxygenated blood has no effect. Using pharmacological and genetic approaches, it was also discovered that enzymatic processing of GSNO to cysteinylGlySNO by γ-glutamyl transpeptidase (γ-GT) was required for GSNO to augment minute ventilation, and mice deficient in γ-GT were shown to have a grossly abnormal ventilatory response to hypoxia (101). Together, these data show that endogenous SNOs (likely those derived from deoxygenated rbc) can act at the level of the nTS to mediate the ventilatory response to hypoxia (101) and SNO-based signaling may play a more pervasive role in controlling the drive to breathe, e.g., in carotid body chemoreceptors. In addition to central effects, SNOs can also augment ventilation via bronchodilation (102, 103) and possibly via effects on contractile function of breathing muscles

(e.g., diaphragm, intercostals) (55, 56). These findings also suggest a link between aberrancies in SNO signaling (104) and the disrupted breathing pattern/mechanics (104, 105) that are frequently observed in patients with heart failure.

SNOs and the cellular response to hypoxia versus anemia. Systemic hypoxia (decreased pO$_2$) and anemia (decreased rbc mass and blood Hb concentration) both result in reduced oxygen delivery to tissues. Although both stressors activate HIF-1α, a ubiquitous transcriptional regulator of hypoxic adaptation (1), the mechanism of activation differs. In sustained hypoxia, arterial O$_2$ delivery is reduced due to low pO$_2$ and SaO$_2$. In this setting, inactivation of cellular O$_2$ sensors (in particular, O$_2$-dependent prolyl hydroxylases) results in the stabilization/accumulation of HIF-1α and enhanced transcriptional activity. Canonical HIF-1α targets include erythropoietin, VEGF, and GLUT1, which regulate erythropoiesis, angiogenesis, and glucose utilization, respectively (1, 5). On the other hand, anemia reduces blood O$_2$ content through a reduction in Hb concentration while preserving PaO$_2$ and SaO$_2$. Therefore, while both hypoxia and anemia are associated with reduced O$_2$ delivery, the relative sparing of SaO$_2$ during anemia fails to trigger classical O$_2$-dependent HIF-1α signaling (5). Rather, HIF-1α activation during anemia occurs because S-nitrosylation — generating SNO-pVHL, SNO-PHD2, and SNO-HIF-1α — serves to activate HIF-1α under normoxic conditions (106–109). In addition, GSNOR abundance has been found to decrease in rodent models of acute anemia, which could further augment SNO bioactivity and hypoxic adaptation (110). Notably, endogenous SNOs are critical for ischemic cardioprotection in mouse models (35). Inasmuch as anemia is a robust predictor of adverse outcomes in patients with ischemic heart disease (111–113) and heart failure (114), these experimental data strongly suggest that aberrant SNO-based signals can mediate the detrimental effects of anemia in these clinical settings and suggest new therapeutic approaches.

Therapeutic potential of modulating S-nitrosylation
Decreased levels and/or impaired bioavailability of SNO-modified proteins have been observed in a variety of disease states characterized by tissue hypoxia (5), including congestive heart failure (98). To the extent that NO donors (e.g., isosorbide dinitrate, nitroglycerin) have beneficial effects in patients with heart failure and ischemic heart disease (115–118), these therapies may function, in part, via modulating S-nitrosylation of key myocardial proteins (e.g., RYR2, HIF-1α, and β_2-AR signaling components including GRK2). More efficient methods of SNO delivery to the myocardium (e.g., low-molecular-weight SNOs, SNO-loaded rbc) may have clinical benefits in this setting (50). In particular, rbc represent an attractive vehicle for SNO delivery: (a) rbc interface with endothelium to form the largest aggregate intercellular interaction in the human body and therefore can exert beneficial effects across multiple organs; (b) rbc deliver both NO bioactivity and oxygen under the control of pO$_2$-based allostery and thereby facilitate autoregulatory control (3); and (c) rbc provide unique access to the microcirculation that is dysfunctional in many cardiovascular diseases. The importance of rbc-mediated SNO delivery is reflected in the clinical observation that anemia is strongly associated with adverse outcome in patients with cardiovascular disease, even with modest levels of blood loss (111–114). Paradoxically, liberal restoration of plasma Hb concentration via standard clinical practices (i.e., transfusion of banked erythrocytes, administration of erythro-

poietin) has failed to demonstrate improved tissue oxygenation or clinical benefit in this setting, and multiple studies indicate that this practice might actually be harmful (119–122). One contributory explanation for this apparent paradox is that banked blood is depleted of SNO and fails to deliver bioactive NO, and can therefore exacerbate tissue hypoxia (123–125). Accordingly, strategies aimed at restoring SNO levels in rbc (96, 126) may represent an attractive strategy to improve NO delivery, myocardial performance, and tissue oxygenation.

Conclusions

O_2 sensing and coupling mechanisms at the cellular, tissue, and integrated system levels involve critical roles for *S*-nitrosylation–based signaling, a ubiquitous and evolutionarily conserved mechanism for control of cellular function. In particular, SNOs have been shown to regulate the activities of ion channels, receptors, respiratory proteins, and enzymes that ultimately transduce hypoxic signals into increased alveolar ventilation, matched alveolar perfusion, augmentation of cardiac and skeletal muscle performance, and enhanced microcirculatory blood flow. Dysregulation of the cardiovascular system in heart failure can be understood in these terms to represent global impairments of O_2 delivery, equated with reduced bioavailability of SNOs and their hypoxia-mimetic signaling function. Increased understanding of how SNO-based signals execute control of myocardial, skeletal, vascular, and hematologic function will continue to pave the way for new cardiovascular therapeutics.

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