Among vertebrates able to tolerate periods of oxygen deprivation, the painted and red-eared slider turtles (Chrysemys picta and Trachemys scripta) and the crucian carp (Carassius carassius) are the most extreme and can survive even months of total lack of oxygen during winter. The key to hypoxia survival resides in concerted physiological responses, including strong metabolic depression, protection against oxidative damage and—in air-breathing animals—redistribution of blood flow. Each of these responses is known to be tightly regulated by nitric oxide (NO) and during hypoxia by its metabolite nitrite. The aim of this review is to highlight recent work illustrating the widespread roles of NO and nitrite in the tolerance to extreme oxygen deprivation, in particular in the red-eared slider turtle and crucian carp, but also in diving marine mammals. The emerging picture underscores the importance of NO and nitrite signaling in the adaptive response to hypoxia in vertebrate animals.
body temperature, no endotherm species can tolerate being completely anoxic for even short periods of time (9). Diving marine mammals and birds can, however, endure anoxia in some of their tissues (e.g., skeletal muscle) during long dives (the sperm whale *Physeter catodon* and elephant seal *Mirounga leonina* for up to 2 h) (87) while hunting and foraging for otherwise inaccessible food sources in the sea. This astonishing diving behavior is made possible by the massive blood and muscle oxygen stores that are used to effectively sustain aerobic metabolism during most dives (85, 86) to the extent that extreme divers like elephant seals tolerate almost complete depletion of blood oxygen stores during relatively short routine dives (68). When dives exceed the aerobic dive limit, a selective peripheral vasoconstriction redistributes blood flow so that circulating oxygen is secured for critical tissues such as heart and brain, whereas other tissues, such as skeletal muscle, must rely on anaerobic metabolism (8, 70, 85, 92). A curtailed perfusion of skeletal muscle effectively prevents that, during dives, the oxygen contained in the blood is depleted by the high oxygen affinity myoglobin contained in the skeletal muscle. When muscle perfusion resumes upon surfacing, lactic acid produced in the skeletal muscle is released to the blood stream, and elimination of lactate in the liver after long dives increases recovery time at the surface (59). Selective peripheral vasoconstriction and reduced heart rates are key elements in the redistribution of oxygen supply to the most vital organs during dives in marine mammals (85, 87). Strong bradycardia and redistribution of blood flow also occurs in anoxic submerged turtles (82, 102), where blood flow is strongly reduced to digestive organs and kidney, whereas blood flow to liver and shell increases, in line with the use of the shell for buffering and the liver glycogen for fueling anaerobic metabolism. By contrast, cardiac output is largely maintained in the anoxic crucian carp, probably because of the necessity of excreting ethanol through the gills (103).

**Central Problems of Hypoxia/Anoxia and Subsequent Reoxygenation—and Their Solution**

When oxygen supply and oxidative ATP production is curtailed, a typical vertebrate cell quickly experiences cellular depletion of ATP, since glycolysis cannot sustain a sufficient rate of ATP production for long. The decline in cellular ATP leads to failure of ion-transporting ATPases to maintain ion gradients across cell membranes, resulting in membrane depolarization, cytosolic Ca$^{2+}$ overload, and other destructive events that trigger cell death in critical organs, such as the brain or the heart (9, 66). Reoxygenation is also critical, as a restart of the mitochondrial respiratory chain after a period of inhibition leads to massive production of reactive oxygen species (ROS), in particular superoxide (O$_2^-$) and its product hydrogen peroxide H$_2$O$_2$, causing further cellular injury via protein and DNA oxidation and lipid peroxidation (60). This chain of events is similar to what occurs in the ischemiareperfusion injury, where tissue is damaged first by oxygen lack and then by sudden reoxygenation when blood flow is resumed after myocardial infarction or stroke (60, 73). Any successful adaptation to anoxia and reoxygenation accordingly needs to tackle these central problems.

One key adaptation observed in hypoxia-tolerant species is the ability to undergo reversible metabolic depression, where a low ATP production is at balance with a low ATP consumption to prolong energy reserves (9). During winter anoxia, freshwater turtles and crucian carp depress their metabolic rates much beyond the level expected from low temperatures per se (49, 80). This involves key features such as “channel arrest” (41), where a reduced cellular ion permeability allows reduced ATP expenditure on ion pumping and downregulation of protein synthesis (7, 9, 71). In this way, cellular ATP can be prevented from falling to critically low levels, even though glycolysis is the only ATP-generating pathway, and membrane potential is stabilized. In the anoxic brain, moreover, inhibition of spontaneous electrical activity (“spike arrest”) induced by the neurotransmitter GABA contributes to reduce energy consumption further (10, 78, 83). By these mechanisms, anoxic turtles and crucian carp maintain normal ATP levels in the brain (80), whereas other tissues attain lower but sufficient steady-state ATP concentration (101). Metabolic depression is most likely less pronounced in diving mammals, although even a small decrease in body temperature of 2–3°C during diving may still decrease metabolic rates to an appreciable extent (87). However, because of their cryptic lifestyle, much remains to be known in these elusive species.

An enhanced defense against oxidative stress and tissue injury at reoxygenation is another key factor for surviving anoxia and reoxygenation. Carp and freshwater turtles defend themselves against ROS by having high levels of antioxidant enzymes superoxide dismutase and catalase, as well as high levels of glutathione that act as an intracellular redox buffer (39, 88, 104). High levels of glutathione and several antioxidant enzymes have been found in seals (90, 116, 117), and several positively selected genes involved in the metabolism of glutathione have been recently identified in whale genomes (122).
From these observations, the emerging picture is that of a common set of standard physiological responses across hypoxia-tolerant vertebrates, namely depression of oxygen consumption rates, enhanced cytoprotection against ROS injury, and, at least in air-breathing species, redistribution of blood flow in the circulation. This suggests the existence of a master universal signaling molecule that may be at the origin of these responses to control and coordinate adaptive physiological processes at the molecular level. One such signaling molecule is nitric oxide (NO; nitrogen monoxide). Together with its metabolites, in particular nitrite and S-nitrosothiols (SNO; formed when Cys thiols react with NO⁺), NO is today recognized as a critical signaling molecule in the control of the reduction of metabolic rate and regulation of local blood flow during tissue hypoxia and in cytoprotection during ischemia and reperfusion, as shown by a large number of studies performed mainly on humans and mouse models during the past two decades (28, 40, 65, 114). In this review, we provide recent evidence showing that NO and its metabolites have a much broader biological relevance and appear to be essential components of the extreme physiological adaptation to hypoxia of crucian carp, turtles, and likely diving mammals.

**NO Metabolism and Biological Functions**

NO is a gaseous free radical that regulates a wide range of biological processes. It typically exerts its physiological functions by binding directly to ferrous iron of heme proteins [forming iron-nitrosyl (FeNO)] or indirectly (e.g., through N₂O₃) by S-nitrosation of protein thiol groups (forming SNO) (40). NO is synthesized at low physiological levels (121) by NO synthase (NOS) enzymes that catalyze the oxidation of the amino acid L-arginine with molecular oxygen to form L-citrulline and NO through a complex mechanism dependent on several cofactors (2). Three main isoforms of NOS are widely present and were originally named according to the tissues where they were first found or to their mode of expression (40, 58): neural NOS (nNOS or NOS1) present in nerve cells; inducible NOS (iNOS or NOS2), the NOS isoform with the highest rate of NO synthesis expressed in cell types of the immune system in response to infection; and endothelial NOS (eNOS or NOS3) present in the vascular endothelium. Perhaps the best known mode of action of NO is in the vasculature, where Ca²⁺-dependent activation of eNOS in the endothelium (in response to cholinergic stimulation or shear stress from the blood) results in the production of NO, which freely diffuses into nearby vascular smooth muscle to activate its major target in the blood vessels, the heme-containing soluble guanylate cyclase, which generates cGMP and causes vasorelaxation (74). This mode of action is important for tissue blood flow regulation but also maintains a basal dilatary tone in arterial vessels and consequently impacts on blood pressure. Interestingly, in fish–comprising 50% of all vertebrate species—a gene for eNOS has not been identified, and it appears that the eNOS isoform evolved after the divergence of tetrapods and fish (4). NO-dependent vasorelaxation is, however, present in fish and may be mediated by nitrergic nerves expressing nNOS (21). Some fish (e.g., cyprinids like crucian carp) furthermore express different variants of iNOS in different tissues (4, 91).

When NO produced in the endothelium diffuses into the blood, it rapidly reacts with oxygenated Hb to form nitrate (22, 74). This NO-scavenging reaction is important for keeping NO effects local. NO also reacts with dissolved free oxygen to form nitrite, whereby nitrite (typically present in animal tissues at or below μM concentrations) in the plasma can be conveniently used as a marker of overall NOS activity (57).

The requirement of NOS for oxygen has the inherent complication that de novo NO formation via NOS is compromised under tissue hypoxia. However, over the past decade it has become increasingly clear that NO can be “recycled” from its end-product nitrite and that nitrite functions as a pool of NO availability that is activated in vivo under hypoxic and/or acidic conditions (64, 65) (FIGURE 1). Thus, when the NOS reaction becomes compromised by hypoxia, the NO production is gradually taken over by nitrite reduction (64, 114). A number of proteins and enzymes have been shown to be capable of converting nitrite to NO by acting as nitrite reductases, primarily deoxygenated hemoglobin (16, 23) and myoglobin (96), xanthine oxidoreductase (31), and carbonic anhydrase that functions as a non-redox nitrite anhydrase (1) (FIGURE 1). Endogenous nitrate may play an important role in regenerating nitrite and NO in that it can be converted to nitrite by the ubiquitous enzyme xanthine oxidoreductase but on longer time scales than the nitrite to NO conversion (51). A more efficient way of reducing nitrate to nitrite is provided by commensal bacteria in the oral cavity that rapidly convert nitrate contained in the diet to nitrite, which is then in part absorbed in the gut, from where it enters the blood circulation (64). Thus the seemingly inert inorganic anions nitrite and nitrate are in fact used in the body as natural sources of biologically active NO, particularly during hypoxia.

As discussed in detail below, the physiological relevance of the nitrite to NO conversion varies among tissues, as summarized in FIGURE 2. In the
blood, the NO generated from nitrite by deoxygenated hemoglobin may target soluble guanylate cyclase in the vasculature, thereby increasing vasodilation and blood flow in hypoxic tissues. In the hypoxic heart, nitrite targets primarily mitochondria, where it inhibits cytochrome c oxidase (complex IV) via generation of NO by deoxygenated myoglobin, and complex I activity by S-nitrosation. The physiological consequences of these interactions are to limit oxygen consumption rates of complex IV and to block a major site of ROS generation, namely complex I, respectively. All these nitrite-dependent interactions are potentially underlying basic molecular mechanisms of physiological adaptations that have evolved to survive hypoxia and anoxia.

**Circulation: Controlling Blood Flow and Organ Perfusion**

Ever since the discovery of its physiological role in humans (16), the reaction between nitrite and deoxygenated hemoglobin has attracted particular attention due to its relevance in local blood flow regulation. Nitrite readily permeates the red cell membrane via simple diffusion of HNO2 and facilitated diffusion via the anion exchanger AE1 membrane protein, creating an equilibrium distribution of free (i.e., unbound) nitrite across the red blood cell membrane (53). Inside the red blood cell, nitrite reacts with the ferrous heme of deoxygenated hemoglobin to form NO and oxidized (ferric) heme according to the general scheme (16):

\[
\text{NO}_2^- \quad (\text{nitrite}) + \text{Fe}^{2+} \quad (\text{ferrous heme}) + \text{H}^+ \rightarrow \text{NO} \quad (\text{nitrile oxide}) + \text{Fe}^{3+} \quad (\text{ferric heme}) + \text{OH}^- \\
\]

Ferric heme is subsequently reduced to functional (ferrous) heme in the red blood cell. The degree of hemoglobin saturation with oxygen combined with the nitrite reductase activity of deoxygenated hemoglobin provides an attractive and powerful mechanism by which red blood cells sense local hypoxia in the microcirculation and respond to it by producing NO that induces local vasodilation to reestablish oxygen supply (16, 30, 54). In this process, sufficient NO apparently escapes the red blood cell and reaches the vasculature (16, 19) without being scavenged by oxygenated hemoglobin (to form nitrate) (22) or deoxygenated hemoglobin [to form highly stable nitrosyl hemoglobin (FeNO)] (12). Part of the nitrite-induced vasodilation during hypoxia is due to nitrite reduction taking place inside the vessel wall (3, 20), catalyzed at least in part by myoglobin expressed at low levels in the vasculature of mammalian (109, 110) and fish species (17).

In strongly hypoxic animals, where blood oxygen reserves are progressively depleted, reduction of nitrite to NO in the blood is greatly facilitated by the high levels of deoxygenated hemoglobin, and in the anoxic turtle (50, 56) and crucian carp (91) by the elevated nitrite levels inside red blood cells. Supporting this view, very high levels of nitrosyl hemoglobin FeNO (a typical signature of NO generation in low oxygenated blood) have been detected in the blood of anoxic turtles (50, 56).
Furthermore, hypoxia-tolerant species, including turtle (50), carp (52), and harbor porpoise (55), appear to have hemoglobins with higher nitrite reductase activity compared with hypoxia-intolerant ones, such as trout (55) (FIGURE 3). Interestingly, when data from different species are compared under similar experimental conditions, there is a clear increase in the nitrite reductase activity as the oxygen affinity of the hemoglobin increases (FIGURE 3), similar to what is observed for mutant or modified human hemoglobin (19). This effect can be ascribed to the T-R allosteric equilibrium of the hemoglobin between low-affinity (T state) and high-affinity conformation (R state), since hemoglobins with a high oxygen affinity are typically more shifted toward the R state (120). Due to decreased heme redox potential or improved accessibility of nitrite to the heme pocket, hemoglobin in the R state has a higher nitrite reductase activity than in the T-state conformation (42). Interestingly, the

**FIGURE 2.** Origin, fate, and physiological roles of nitrite in the blood and heart during hypoxia

A: in the oxygen-deprived blood, plasma nitrite enters the red blood cells through the anion exchanger membrane protein AE1 or by simple diffusion as protonated nitrous acid. Once in the red blood cell, nitrite reacts with deoxygenated hemoglobin to generate NO, which freely diffuses out through the red blood cell membrane and the endothelium, and activates soluble guanylate cyclase (sGC) in the vessel smooth muscle cell. NO may also bind to vacant heme in deoxygenated hemoglobin to generate FeNO complex or react with any oxygenated hemoglobin present to generate nitrate (not shown). Active sGC in the vasculature then converts GTP to cGMP, which induces relaxation. Plasma nitrite may also diffuse into endothelial cells, where it may react with deoxygenated myoglobin contained at low levels and generate NO, which induces relaxation. Thus blood vessels dilate as a response to increased nitrite availability and to decreased oxygenation in the blood and within the vessel. B: in the hypoxic heart, nitrite accumulates in the cardiomyocytes of crucian carp and turtles, possibly as a result of increased NOS expression or as an enhanced transfer of nitrite from extracellular to intracellular compartments. Within the cardiomyocyte, nitrite reacts with deoxygenated myoglobin to generate NO, which diffuses into mitochondria and decreases oxygen consumption rate by binding to the heme of cytochrome c oxidase (complex IV of the electron transport chain), thereby contributing to metabolic suppression during hypoxia. This process is reversible, and NO is displaced from complex IV when oxygen is again available. Under tissue hypoxia, nitrite may also form N2O3, which may react with a specific Cys residue on complex I to generate a labile SNO derivative. S-nitrosation of complex I limits ROS formation formed at reoxygenation, when highly reduced complex I and III (not shown) of the mitochondria react with oxygen. This process acts to protect the heart and other organs against excessive oxidative stress after hypoxic episodes.
same adaptive mechanism of the hemoglobin that improves efficient oxygen loading in ambient hypoxia (i.e., a conformational shift of the allosteric equilibrium toward the high-affinity R state) (120) appears to favor at the same time faster NO production from nitrite and thereby efficiently blood supply to hypoxic tissues. Monomeric myoglobin has a higher oxygen affinity than hemoglobin and is also a faster nitrite reductase (FIGURE 3). As for the hemoglobin, available data on myoglobin from different species show a direct correlation between oxygen affinity and nitrite reductase activity (FIGURE 3), which is related to changes in heme reactivity (37).

In exercising humans, the vasodilatory effect of NO generated from nitrite in the red blood cells by deoxygenated hemoglobin enhances blood flow to hypoxic exercising muscles and decreases peripheral resistance (16). However, in anoxic turtles and in diving mammals, a strong adrenergic tone is present that constricts a large part of the peripheral systemic circulation to the skeletal muscle, acting to maintain blood pressure to most vital organs in the face of very low heart rates. It is possible that, under conditions of a strong peripheral vasoconstriction in the circulation, NO produced from nitrite in the oxygen-depleted blood could favor dilation of the large conducting arteries and provide room for the blood volume expelled from the contracting peripheral vessels. Thus nitrite-derived NO may help redistribute blood volume during prolonged hypoxia and anoxia. In support of this conclusion, topical application of a NOS inhibitor prolonged hypoxia and anoxia. In support of this conclusion, topical application of a NOS inhibitor or NO donor in the brain of anoxic turtles has no effect on the cerebral blood flow (43), suggesting that nitrite-derived NO may already elicit maximal vasorelaxation.

**Elevated Levels of Nitrite in Hypoxia-Tolerant Species**

At normal oxygen levels, a number of fish species (flounder, eelpout, oyster toadfish, brown trout) have plasma levels of nitrite similar to those of mammals (~0.2 μM), whereas slightly higher levels (0.75–1.75 μM) have been reported in hypoxia-tolerant species like goldfish and crucian carp (33, 55, 91). Elevated normoxic plasma nitrite concentrations have also been observed in red-eared slider turtles, even though values vary between studies, possibly as a consequence of differences in acclimation temperatures (50, 56). The origin of these higher nitrite plasma levels in hypoxia-tolerant vertebrate species is not entirely clear, although it may reflect a high overall NOS activity or expression under normoxia (57). Interestingly, transcription of iNOS and eNOS is under control of the hypoxia-inducible factor transcription factor HIF-1α (18, 69), which is stabilized when cells become hypoxic (75, 93), and conversely nNOS activity is important for HIF-1α stability in the brain (111, 112). Stabilization of HIF-1α factor may occur in crucian carp even under normoxic conditions, suggesting a high basal expression of hypoxic-regulated genes, including iNOS (100). Freshwater fish, including the crucian carp, may also take water nitrite up through the gills, which provides an alternative route for boosting internal levels (55).

The trend of higher circulating levels of nitrite in hypoxia-tolerant than hypoxia-intolerant animals may also apply to diving mammals, as observed in the only species investigated so far, the harbor porpoise (99). The high nitrite levels secure increased NO bioavailability during hypoxia. Interestingly, elevated circulating nitrite is also a viable strategy for dealing with hypoxia in humans. Tibetan highlanders have considerably higher plasma levels of nitrite (~12 μM) and other bioactive NO products (nitrate, SNO) than lowlanders, which are associated with higher blood flow to compensate high-altitude hypoxia (25) and may originate from a constitutively high NOS expression (6). Circulating nitrite levels also increase during human acclimatization to high altitude but not to the same extent as found in Tibetan highlanders (6, 63). Although the

![FIGURE 3](http://physiologyonline.physiology.org/)
inspired oxygen tension for Tibetans at an altitude of some 4,200 m is ~80 Torr (compared with ~150 Torr at sea level), aquatic animals can be faced with even lower values. After acclimation of goldfish to hypoxia for 2 days to water oxygen tensions of 29 and 19 Torr (at and below the critical oxygen tension of goldfish, respectively), nitrite and nitrate levels decrease in the plasma but remain essentially constant in tissues, suggesting reduced NOS activity, as expected from the lower oxygen availability and concomitant nitrite transfer from extracellular to intracellular compartments (33). In crucian carp exposed to anoxia, plasma nitrite and nitrate also decrease, whereas nitrite levels in the heart dramatically increase from ~1 μM in normoxic specimens to some 10 μM after 5 days of anoxia (91), again suggesting that plasma nitrite is transferred into cardiomyocytes. Within the heart, nitrite is used in part to produce NO and in part S-nitrosoate cellular targets (likely via N₂O₃), as reflected by major increases in iron-nitrosyl (FeNO) and S-nitrosothiols (SNO) during anoxia exposure (91). In other tissues such as brain, gill, liver, and white skeletal muscle, nitrite remains relatively constant under anoxia but is still metabolized to FeNO and SNO in a tissue-specific manner (91). The strategy seems to be general for anoxia-tolerant vertebrates. Thus anoxic red-eared slider turtles also elevate nitrite, FeNO, and SNO in the heart, whereas nitrite is maintained and FeNO and SNO are increased in brain and liver (56). These changes are gradually reversed upon reoxygenation (56).

Interestingly, anoxic turtles also develop elevated nitrite concentrations in pectoral muscle composed of both red and white fibers (56), whereas anoxic crucian carp acquire a small decrease in nitrite in white skeletal muscle (91). Given the increase in cardiac nitrite in both species, it appears that a high myoglobin and mitochondria content (as in cardiac and red muscle fibers) is important for increasing cellular nitrite. Anoxic crucian carp also show an increased expression of a specific iNOS isoform in anoxia (91). We speculate that, in the transition from normoxia to anoxia, while there is still sufficient oxygen for enzymatic NOS activity, iNOS upregulation would generate NO that may oxidize to nitrite and accumulate in the heart. When anoxia is fully established, however, iNOS will not be able to produce NO, and the NO cannot autoxidize to nitrite (since both processes require oxygen). Cellular nitrite may also originate from transfer of nitrite from extracellular to intracellular compartments during hypoxia/anoxia, as supported by data from goldfish, crucian carp, and turtles (33, 56, 91). The extracellular reservoir is, however, not sufficient to maintain or increase tissue nitrite concentrations during long periods of anoxia, unless some additional reservoirs are available. We are currently investigating the hypotheses that crucian carp utilize ambient nitrite that is taken up across the gills and that turtles exploit nitrite reservoirs in the shell that become mobilized during anoxia, much in line with the release of calcium carbonates for buffering (56, 91). Finally, nitrate, which is abundant in the tissues of normoxic crucian carp (60–80 μM) and turtle (100–400 μM) (56, 91), may also contribute to nitrite generation during anoxia. Even if the enzymatic conversion of nitrate to nitrite is slow in mammals (51), and presumably also in ectotherms at low temperatures, this process may become physiologically important during the long anoxia adaptation experienced by these species. An involvement of oral or gut bacteria in the nitrate to nitrite reduction during winter dormancy appears unlikely, since animals do not eat when strongly hypoxic or anoxic.

Cellular Roles of Nitrite and NO: Reduction of Metabolism and Protection From Oxidative Stress

Under conditions where the heart becomes hypoxic, nitrite increasingly reacts with deoxygenated myoglobin generating NO, according to the same reaction scheme described above for hemoglobin. A major target of the NO generated in the heart under these conditions is the cytochrome c oxidase (complex IV) of the mitochondria (14, 27). In tissues that do not express myoglobin, the nitrite conversion to NO can be catalyzed by other enzymes, such as xanthine oxidoreductase or carbonic anhydrase (FIGURE 1). Because of its competition with oxygen, NO is an effective inhibitor of respiration rates when oxygen levels are low (15). Thus, during hypoxia, NO inhibition of complex IV may contribute to metabolic depression in tissues and to prolong oxygen availability (96).

It has been shown in hypoxia-tolerant species goldfish (84) and turtle (72) and hypoxia-intolerant trout (84) that inhibition of mitochondrial respiration by NO (derived from either nitrite or NOS) in the hypoxic myocardium occurs without major changes in contractility. Thus myocardial efficiency (i.e., the force generated per oxygen consumed), increases, and this effect of NO may be of particular importance in maintaining myocardial contractility when the heart becomes hypoxic or anoxic (103). Hypoxic goldfish hearts even show an enhanced mechanical performance compared with normoxic ones (44). An increased myocardial efficiency in hypoxic fish or turtles could possibly be due to increased mitochondrial coupling by NO or to enhanced glycolytic supply of ATP to contractile elements. In this respect, it is of interest that
dietary nitrate has been found to reduce oxygen cost during physical exercise in humans (5, 62), which appears consistent with an improved mitochondrial efficiency, increasing the amount of ATP produced per oxygen consumed (61). The effect relates to the metabolism of nitrate to nitrite and NO over time, and involves signaling mechanisms (possibly triggered by NO binding to cytochrome c oxidase) that significantly reduce H+ leakage across the inner mitochondrial membrane (61).

Given the comparatively elevated circulating nitrite and nitrate concentrations in harbor porpoise (99), it is possible that a similar NO-dependent increase in mitochondrial coupling may increase the efficiency of skeletal muscles in diving mammals, thereby contributing to enhancing diving performance and to reducing rates of oxygen consumption during dives. Although, in the whale myoglobins investigated so far, nitrite reductase activity does not correlate with average dive duration (36), in vivo rates of nitrite reduction to NO would be accelerated by the far higher myoglobin concentration present in the muscle of whales capable of longer dives (36). Again, the same molecular mechanisms allowing whales to enhance oxygen storage capacity in the muscle tissue would also enhance muscle efficiency when these oxygen stores become depleted. Interestingly, new data show that erythropoietin, whose production is also dependent on HIF-1α (94), increases myoglobin concentration in human skeletal muscle (32). Whether this applies to diving mammals regularly experiencing internal hypoxia is an intriguing possibility for future investigations.

Another main target of nitrite and NO in hypoxic and anoxic tissues is complex I of the mitochondrial respiratory chain (FIGURE 2). The acidic conditions present in tissues during hypoxia and anoxia and the relatively high concentration of nitrite favor generation of N₂O₃, which is a strong S-nitrosating agent. Several mammalian studies have shown that moderate elevations of nitrite are associated with reduced cell death and infarct size following myocardial ischemia and reperfusion (24, 38, 95, 119). Complex I is S-nitrosated at a specific Cys residue present in the mammalian protein (13), and this labile posttranslational modification appears central for limiting oxidative damage during reoxygenation. The inhibition of complex I by S-nitrosation leads to a slow restart of the electron transport chain when oxygen is reintroduced, which strongly reduces ROS production and ROS-induced damage (11, 13, 97). Interestingly, electron flow from complex I to II is reduced in cardiac fibers from anoxic turtles (29), suggesting that complex I may indeed be inhibited. S-nitrosation of a number of other proteins also contributes to cytoprotection. For example, S-nitrosation decreases activity of caspases, which are key regulators of apoptosis, and inhibits cell death (45), whereas S-nitrosation of the L-type Ca²⁺ channel and the Ca²⁺ ATPase (SERCA2a) alleviates problems with cytosolic Ca²⁺ overload during anoxia and early reoxygenation in the heart (105, 106). Anoxic turtles show a strongly reduced activity of mitochondrial F₁F₀-ATPase (complex V), which limits ATP consumption during anoxia by inhibiting the reverse mode (ATP-consuming) action of F₁F₀-ATPase (29). This beneficial effect may also be mediated in part by S-nitrosation, which inhibits F₁F₀-ATPase (105).

It is likely that nitrite-linked NO and SNO formation contributes to cytoprotection not only in the heart but also in other tissues, but the potential targets of S-nitrosation may differ between tissues. In the anoxic brain, the N-methyl-D-aspartate (NMDA) neuronal receptor is preferentially inhibited by S-nitrosation under low-oxygen conditions (107). The NMDA receptor of neurons is strongly inhibited during anoxia in the turtle brain, where it plays a central element in depressing neuronal activity ("channel arrest") and in preventing Ca²⁺ influx and cellular death (7). However, whether S-nitrosation contributes to this inhibition remains to be confirmed.

A seemingly unique SNO-dependent cytoprotective mechanism is operating in the heart of rainbow trout and salmon that are able to tolerate internal hypoxia during intense swimming but not ambient hypoxia. In these species, S-nitrosation of heart myoglobin at a specific Cys residue is allosterically linked to oxygenation (34, 35), whereby low oxygen levels in the heart would promote NO or SNO release from myoglobin, possibly to improve heart efficiency during intense activity, when the heart becomes hypoxic (84). Reactive cysteines are generally absent in mammalian myoglobins, including diving mammals, whereby this particular type of regulatory control modification cannot take place.

Conclusions and Perspectives

Although much remains to be investigated, there are clear indications from studies on nonmammalian species that hypoxia tolerance is tightly associated with enhanced circulating and tissue reserves of nitrite that are selectively directed into NO- or SNO-generating pathways to help in controlling blood flow, in limiting oxygen consumption, and in cytoprotection against oxidative damage. Interestingly, the range of NO modes of generation and targets may become even more complicated in vivo by the presence of other signaling molecules, including hydrogen sulfide and carbon monoxide, with overlapping functions in
the control of respiration and vasodilation (26, 81). Recent studies have reported altered levels of hydrogen sulfide in hibernating brown bears (89) and of carbon monoxide in the northern elephant seal (108). Much can still be learned by studying how anoxia-tolerant species handle anoxia and reoxygenation, and we hope this short overview will stimulate intensified research into these animals to uncover their secrets, which also could include mechanisms of therapeutic relevance in a human setting.

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