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Hypoxia and Anoxia Tolerance of Vertebrate Hearts: An Evolutionary Perspective

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Abstract

Extreme changes in environmental oxygen (O_2) is a constant issue that ecthotherm vertebrates have to deal with, whereas for endotherms severe hypoxia and reoxigenation are usually related to a pathological state. The physiological mechanisms of hypoxia tolerance in echtotherms are based on biochemical evolutionary adaptations and may serve in understanding endogenous phenomena of protection against diminished O_2 availability in the heart. In this review, we will, therefore, describe different species of fish, amphibian, and reptile that are well-known examples of cardiac tolerance to O_2 deficiency. We will then focus on a subset of Antarctic fishes which have lost physiological transporters of O_2 such as hemoglobin and myoglobin (Mb) and that have reached a surprising adaptation to this extreme environment. Moreover, we will concentrate on the cardio-protective effects of the interaction between Mb and nitric oxide with particular emphasis on the nitrite-reductase function of Mb. Finally, the role of a recently described gasotransmitter, the free diffusible hydrogen sulfide, will be briefly discussed in relation to hypoxia. This evolutionary and comparative perspective may provide a useful and heuristic stimulus for medically oriented research aimed at elucidating the environmental and genetic risk factors underlying the vulnerability of the human heart. *Antioxid. Redox Signal.* 14, 851–862.

Introduction

This synopsis will use in a comparative vertebrate perspective some hypoxia- and anoxia-tolerant hearts as paradigms to illustrate biochemical and physiological strategies for solving problems of oxygen (O_2) limitation or lack of oxygen. In contrast to the endotherms (birds and mammals), many ectotherm vertebrates face even extreme changes in environmental O_2. Studies on their wide range of biochemical and physiological adaptations provide insights on fundamental mechanisms underlying the natural cardio-protection against often astonishing long periods of severe hypoxia or anoxia that are not otherwise detectable in "standard" model organisms, such as the rat. In the first part of this article, we will discuss few examples of fish, amphibian, and reptile species that are well-known champions of cardiac tolerance to O_2 lack. Further, we will consider other species, such as a subset of Antarctic fish, the icefishes, which, due to their evolutionary lost of haemoglobin (Hb) and cardiac myoglobin (Mb), have acquired the characteristics of human disease (extreme anemia with white blood and "pale" heart). However, surprisingly, the "disease" trait represents a feature that has contributed to the animal fitness in its particular frigid habitat. Conceivably, studies on these "evolutionary mutant models" have the potential to identify presently unknown genes and gene/environment interactions that influence human health and underlie human disease, including ischemic cardiomyopathy. In addition, the cardio-protective interactions between Mb and nitric oxide (NO) will be highlighted with emphasis on the novel function of Mb to act as nitrite-reductase. Again, fish and amphibian heart models provide insights on "ancestral" functions of nitrite-NO-Mb processes in vertebrates, which, in turn, may help to expand their actual significance in human cardiac physiology. Finally, the cardioprotective influence of a new member of the gasotransmitter family, that is, the free diffusible hydrogen sulfide (H_2S), will be briefly discussed in an evolutionary context.

We believe that this evolutionary and comparative framework offers a useful and heuristic stimulus for medically oriented research aimed at understanding environmental and genetic risk factors underlying the vulnerability of the human heart.

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Anoxia and Hypoxia Tolerance of Vertebrate Hearts

Environmental stress response and phenotypic plasticity

In contrast to the majority of the warm-blooded avian and mammalian species, most ectotherm vertebrates exhibit a wide range of biochemical and physiological adaptations to face even extreme changes in abiotic environmental factors such as temperature, pH, salinity, and O_2 availability.

Of note, some of these abiotic factors perturb homeostatic processes in the same direction. For example, both cold and hypoxia decrease metabolic requirements (demand-side bioenergetics) and energy production (supply-side bioenergetics). In some species, cardiac anoxic tolerance can be linked to inherent ability of the heart muscle to resist the negative contractile influence of hydrogen ions (31). Therefore, as hypothesized by Hochachka back in 1986 (39), the organism's response to these stressors may involve universal mechanisms. During hypoxia or hypothermia, the maintenance of energy charge, ionic balance, and substrate stores requires that energy conservation (ion channel arrest and metabolic arrest) accompany lowered energy production. Evidence obtained over the past two decades validated the Hochachka's framework. In fact, studies based on postgenomic techniques begin to offer new and detailed insights into the mechanisms underpinning the environmental stress response, including phenotypic plasticity linking hypoxia- and hypothermia-relevant phenotypes. The available data point to a number of genes involved in cellular homeostasis, including energy charge, ATP and protein turnover, and stress protein production (40). These genes respond to various stressors, probably being part of a more general stress response common to many species (18).

Two major physiological adjustments allow to face environmental stresses, namely, organisms either maintain normal levels of activity or of homeostatic potential (capacity adaptation) or enhance resistance to the potentially debilitating or lethal effects of environmental extremes (resistance adaptation) (17). In any case, the stress response is a combination of key individual factors, that is, the appraisal of the environmental change and the ability to cope (7). Laboratory studies in simple environments suggest that, similar to mammals and even humans, divergent stress strategies are activated in echtothermic vertebrates exposed to stress. These strategies, which require differentiated neuroendocrine and hypothalamus-pituitary-adrenal axis responses, are known as "proactive" and "reactive styles." The first is characterized by high locomotor activity and sustained sympathetic activity, the second is characterized by immobility and low sympathetic activity (78). For example, in rainbow trout exposed to hypoxia, the proactive style, which corresponds to an escaping and nonsurviving fish, shows strenous avoidance behavior resulting in an impressive energy expenditure and immediate activation of anaerobic metabolism. Contrarily, the reactive fish remains calm and survives, postponing anaerobic threshold activation (67 and references therein).

The adaptive responses may have life-long consequences if the animal has been exposed to environmental fluctuations during development, or are usually reversible if made during juvenile or adult life. O_2 deprivation signaling in the Zebrafish embryo (a universal model of vertebrate morphogenesis) induces developmental arrest characterized by cell division block in the G and S phases of the cell cycle (63). As a general trend, particularly analysed in frogs, embryonic and neonatal vertebrates are more hypoxia-tolerant than adults (19).

Hypoxia tolerance: unity and diversity in adaptive strategies

Since fish, amphibians, and reptiles are able to survive complete O_2 lack for prolonged periods, it is not surprising that they comprise the most anoxia-tolerant vertebrate species. As reviewed by Driedzic and Gesser (21) and more recently by Bickler and Buck (5), three fundamental adaptations contribute to this capacity: profound metabolic suppression, tolerance of pH and ionic disturbance, avoidance and/or repair of free-radical-induced cell injury during reoxygenation. In addition, long-term anoxic survival includes large stores of glycogen in critical tissues, drastic metabolic depression, and cooperativity in metabolic interactions between different organs and tissues. The latter allows to extend the anoxia tolerance to the whole organism (39).

Among the vertebrates, teleost fish exhibit the highest number of species and interspecific variation. Many groups have successfully evolved within habitats in which their tissues experience the full effects of the ensuing fluctuations in environmental O_2. Consequently, they display some of the most powerful responses for tolerating anoxia or hypoxia, this capacity being usually correlated with environmental O_2 availability and, at the same time, with the anaerobic potential of the organism. Such striking anaerobic tolerance is epitomized by the observation of Mathur back in 1967 (54), who showed that a cyprinid fish (*Rasbora*), which inhabits pools, small ponds, ditches, and streams of India, is able to survive in a sealed jar for more than 100 days.

The prolonged anoxic tolerance sustained by a powerful anaerobic potential requires coping with acidosis associated with anaerobic end-product accumulation (*e.g.*, lactate). Cyprinoid fish, such as the common carp, *Cyprinus carpio*, are able to adjust to seasonal variations in temperature from $<4^{\circ}C$ up to $>38^{\circ}C$ and water oxygen saturations down to just a few per cent of saturation. As a result, the common carp and its cousin, the crucian carp (*Carassius carassius*), have become favored subjects for analysing the physiological responses to environmentally or experimentally induced hypoxia. A notable metabolic depression (30% of normal), which permits glycogen stores conservation, appears crucial for their anoxic survival (52–56). An important part of these studies concerns the mechanisms underlying the anoxic resistance of their hearts, characterized by striking interspecific differences. In fact, the common carp survives 24 h of severe hypoxia but greatly depresses cardiac activity, whereas the crucian carp maintains normal cardiac performance and autonomic cardiovascular control for at least 5 days of anoxia at $8^{\circ}C$ (75). Consequently, the sustained cardiovascular activities allow an efficient perfusion of the liver and the gills. Liver perfusion permits glucose distribution from the unusually large hepatic glycogen store of the crucian carp to metabolically active tissues as well as the transport of waste lactate to the myotomal muscle and its convertion to ethanol (56). Noteworthy, ethanol avoids the problems of acidosis and is, hence, less detrimental than lactate, as first revealed in vertebrates by Shoubridge and Hochachka (69). On the other hand, the efficient perfusion of the branchial vasculature permits the

rapid excretion of ethanol, which is freely diffusible through gill epithelia. Thus, a preserved cardiac performance plays a major role in the metabolic and physiological cooperativity between different organs for extending the anoxic tolerance to the organism as a whole. Figure 1 depicts an example of metabolic interactions between different tissues that minimize lactate accumulation in goldfish (38). Figure 2 illustrates an example of intracardiac metabolic cooperativity in bluefin tuna heart ventricle. The trabeculate subendocardial layer (spongy layer), mainly supplied by venous blood, exhibits higher ability to metabolize lactate in comparison to the subepicardial compact outer myocardial layer exclusively supplied by the coronary oxygenated blood flowing from the gills (29, 20, and references therein).

The role of extracellular glucose and cardiac glycogen in anoxic tolerance has been investigated in the eel (*e.g.*, European eel, *Anguilla anguilla*, and American eel, *A. rostrata*) whose teleosts are also characterized by a remarkable anoxic resistance. In the isolated and perfused heart of the American eel, an exogenous glucose supply is necessary for maintaining force/pressure development during 2 h of anoxia (2). Endogenous glycogen stores are also utilized if oxidative phosphorylation is blocked by NaCN contained in the medium. In addition, these authors assessed the importance of extracellular Ca^{++} on twitch force generated by anoxic ventricular strips.

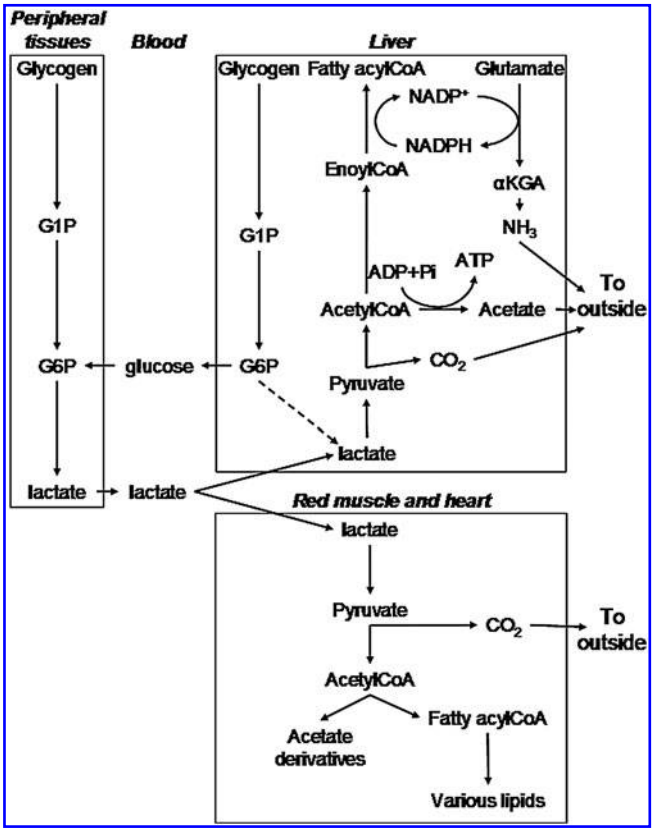

FIG. 1. Hypoxia metabolism in goldfish. The scheme illustrates metabolic cooperativity between different tissues that minimize lactate accumulation. Carbon dioxide, ammonia, and ethanol (or some metabolic derivatives of acetate) are released to the outside as waste anaerobic end products. [reproduced from ref. (36)]. NADP, nicotidamide adenine dinucleotide phosphate; NADPH, nicotinamide-adenine dinucleotide (phosphate) dehydrogenase.

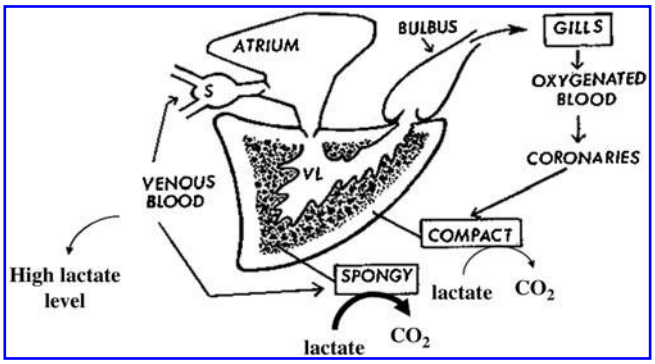

FIG. 2. An example of intracardiac cooperativity in tuna heart ventricle. The subendocardial layer (spongy layer), mainly supplied by venous blood, exhibits higher ability to oxidize lactate to CO_2 in comparison to the subepicardial compact myocardial layer exclusively supplied by the branchial coronary oxygenated blood. Lactate oxidation is measured by the release of $^{14}CO_2$. Lactate accumulation is higher in the spongy (103.8 ± 12.0 μmols/100 mg muscle protein) than in the compact (74.7 ± 13.9 μmols/100 mg muscle protein) layer [modified from ref. (29)].

Studies in mammalian heart preconditioning revealed the major role played by the ATP-sensitive potassium (K_{ATP}) channel-dependent mechanism. The sustained anoxia-resistant cardiac activity of goldfish and carp appears also related to the activation of myocardial K_{ATP} channels (14); however, the identity and importance of this mechanism in comparison with the mammalian counterpart remain to be elucidated (31).

Cardiac survival under prolonged anoxia can be also attained by a drastic downregulation of oxidative phosphorylation (5), as typically exemplified by the goldfish (*C. auratus*) and freshwater turtles (genera *Chrisemys* and *Trachemys*) such as the red-eared turtle (*Trachemys scripta*). These reptiles are capable of astonishing long periods (months) of anoxia tolerance, which, however, is accompanied by drastic suppression of cardiac activity and autonomic cardiovascular control and correlates to environmental temperatures (37). In *T. scripta* exposed to warm temperatures, intrinsic heart rate is 25%–34% lower during anoxia than normoxia. This response, achieved with the contribution of cholinergic cardiac inhibition (36), is facilitated by cold temperature acclimation. In fact, in warm- and cold-acclimated turtles, anoxic bradycardia reduces cardiac and power outputs by 4.5- to 20-fold after 6 h and 14–21 days of anoxic exposures, respectively (see 74 for references). At low temperatures, autonomic cardiovascular control is blunted during anoxia and does not account for the anoxic bradycardia (37) [see ref. (74) for references]. Accordingly, other factors such as anoxia *per se*, acidosis, intrinsic electrophysiological properties of cardiac cells are required (74). The depressed cardiac activity during anoxia sustains the reduction of whole-animal metabolic rate (MR) and blood flow requirement (36), ensuring a low myocardial ATP demand that is below the cardiac glycolytic capacity to supply ATP (23).

An additional mechanism which, in presence of a blunted cholinergic cardiac inhibition, contributes to the very high anoxia tolerance of *T. scripta* at low temperatures is represented

by channel arrest, that is, the reduction in the density and/or activity of ion channels. This was proposed to play a role in decreasing the energetic cost of ion pumping during anoxia (39), particularly during cold-acclimation (74). In the turtle, the ventricular ion current reduction consequent to channel arrest affects the electrophysiological properties of the heart (73) and is responsible for the reduced ventricular excitability and/or the delayed blockage of electrical impulses through the atrial-ventricular node observed in isolated anoxic hearts (42). In addition, ion channel downregulation contributes to ATP preservation because of the lower ATPase activities (*i.e.*, Na+/K+-ATPase) required to restore ion balance. However, the reduction of cardiac ion currents is not universally used by anoxia tolerant species. For example, no cardiac channel arrest occurs in the crucian carp during prolonged, cold anoxia exposure. Unlike the turtle, this fish does not show the extremely reduced cardiac activity associated with prolonged anoxia, but it maintains an almost normal heart performance for at least 5 days in complete O_2 absence (75), its normoxic ATP demand laying within the cardiac glycolytic capability (23). These differences most likely relate to the diverse anoxia-coping strategies exhibited by these two organisms (23). In fact, opposite to the comatose-like state experienced by the turtle exposed to anoxia, crucian carp continue to swim, although slower than during normoxia (57).

Evolutionary Mutant Models

Evolutionary mutant models may offer important clues for understanding genetic and environmental risks factors underlying human diseases. Among these invaluable, but yet under-utilized, natural tools, the icefishes epitomize a striking example. These teleosts are a subset of the endemic Antarctic fish fauna adapted to live at constant subzero temperatures in the coldest marine environment on Earth. They are unique example of disaptation among adult vertebrates for their loss of Hb and, in some species, cardiac Mb, once considered essential-life O_2-binding chromoproteins (71). Due to Hb lack and since they do not produce red blood cells, the icefish are profoundly anemic but have successfully evolved, thriving in the stably frigid, O_2-rich habitat. Conceivably, these abiotic traits have allowed high tolerance of disaptation, followed by subsequent adaptive recovery based on gene expression reprogramming and compensatory multilevel cardio-circulatory responses (27 and references therein). These include hypervolemia, near-zero hematocrit and low blood viscosity, large bore capillaries, increased vascularity with great capacitance, cardiomegaly with very large cardiac output, high blood flow with low systemic pressure, and systemic resistance. All these adjustments counteract the challenge of hypoxemic hypoxia (due to the Hb-free blood and Mb-free cardiac muscle) by increasing peripheral O_2 transcellular movement for aerobic tissues, including the myocardium. At the same time, at subcellular level (myocytes), the compensation is achieved by an extremely high proliferation of mitochondria that reduces the average path lengths between mitochondria and myofibrils, thereby increasing the area of exchange surface between cytoplasm and mitochondria (59). The analysis of the ultrastructure and aerobic metabolic capacities of the heart ventricles of three Antarctic teleosts with variable expression of respiratory pigments, that is, the (Hb+/Mb+) *Gobionotothen gibberifrons*, the (Hb−/Mb+) *Chionodraco rastrospinosus*, and the (Hb−/Mb−) *C. aceratus,* showed that the percentage of the cell volume occupied by mitochondria, Vv (mit,f) was highest in *C. aceratus* with a dramatic value of 36.53 ± 2.07, intermediate in *C. rastrospinosus* (20.10 ± 0.74), and lowest in the red-blooded species (15.87 ± 0.74) (59). However, surprisingly, such high mitochondrial proliferation in icefish hearts does not increase aerobic metabolic capacity. In fact, no differences among the three species were found in the maximal activities (per gram wet mass of tissue) of several aerobically poised enzymes, including citrate synthase and cytochrome oxidase. The results indicated that the high Vv (mit, f) can be viewed as a homeostatic response well suited to reduce the pathlengths for both the O_2 transcellular movement from the ventricle lumen (lacunae) to mitochondria and its intracellular diffusion throughout the lipid conduits of the membranes (59). Interestingly, the subsequent analysis of Urschel and O'Brien (77) highlighted the selective way by which this mitochondrial proliferation has been exploited by the icefish. That is, the increased mitochondrial density in *C. aceratus* (Hb−/Mb−) hearts, rather than from a conventional biogenetic pathway, results from an increase of organelle size, brought about through a proliferation of the outer mitochondrial membrane without a corresponding increase in inner membrane (cristae) surface density, protein synthesis, or mitochondrial DNA replication. The same study also showed that the mitochondrial remodeling is not a genetically fixed trait in the icefish but, instead, is directly influenced by the expression of Hb and Mb.

In icefish, the low cristae densities encountered is compensated by the high mitochodrial volume, thus the density of respiratory chain proteins per g tissue of oxidative muscle is essentially equivalent between red- and white-blooded fishes. The high mitochondrial densities in the icefish myocardium do not increase their aerobic metabolic capacity [reviewed in Sidell (70)]. The very high O_2 content of Antarctic waters and relatively low absolute MR, added to these unusual myocardial mitochondrial content, allow that O_2 is adequately distributed to tissues, so that the obligatory aerobic metabolism of these animals is sustained. This speaks in favor of cardiac metabolic compensation, allowing to minimize the risk of hypoxia in icefish (71).

In conclusion, the icefish cardio-circulatory and sub cellular myocardial remodelings can be a useful case-study for understanding the hierarchic organization of homeostatic circuits and signal-transduction processes (tissue specific O_2/redox sensing systems, signal transduction pathways such as hypoxia-response systems, hypoxia/redox responsive genes) involved in response to the loss of respiratory pigments. This is depicted in the diagram of Figure 3 that, according to their tentative hierarchy of importance, shows multilevel loops activated at different levels to supply an efficient cellular oxygenation.

The Cardio-Protective Role of NO, Nitrite, and Mb

Due to its strictly aerobic metabolism and continuous contractile activity, the myocardium is one of the most sensitive targets of ischemic/reperfusion (I/R) damage. This is particularly true for the human heart but not for the heart of nonmammalian species, such as the turtle and the crucian carp. The cytoprotective processes that contribute to counterbalance the I/R-induced deleterious effects include the NO-

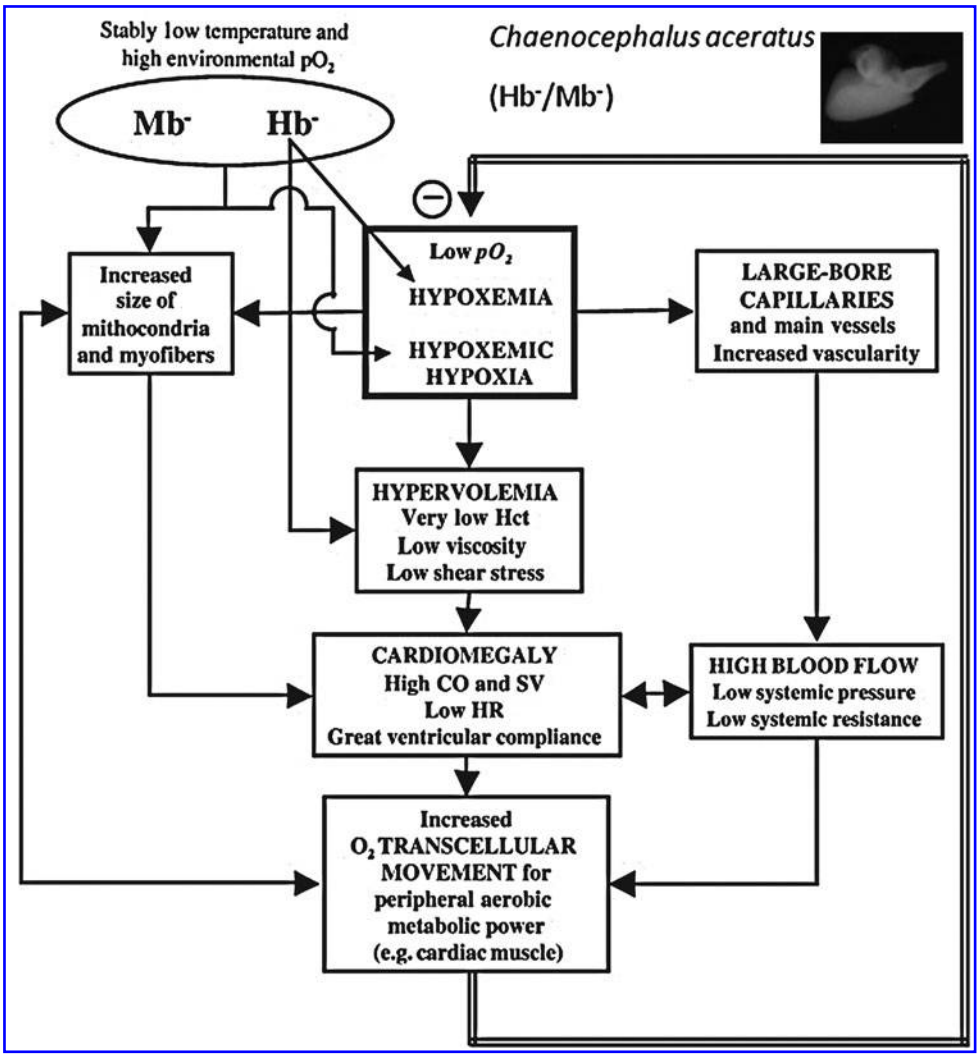

FIG. 3. Putative multilevel diagram showing homeostatic circuits and signal–transduction processes involved to supply an efficient cellular oxygenation in the absence of respiratory pigments. This cascade includes (a) tissue specific oxygen/redox sensing systems (*e.g.*, heme oxygenases); (b) signal transduction pathways (*e.g.*, hypoxia-response systems); (c) hypoxia/redox responsive genes (*e.g.*, VEGF-, endothelial nitric oxide synthase-, HIF-1 genes); (d) phenotypic compensatory responses (circulatory, vascular, and cardiac modifications). The above mechanisms downstream result in adaptative cardio-circulatory readjustments to avoid the risk of hypoxemic and intracellular hypoxia. For references and details, see the text (27). HR, heart rate; SV, stroke volume.

and nitrite (NO_2^-)-dependent activated pathways. An example of these processes is provided in Figure 4.

Intracardiac NO, generated by the diverse endogenous NO synthases (NOS), exerts major paracrine-autocrine myocardial and coronary actions. Under I/R, NO shows a biphasic effect: At higher (*i.e.*, nonphysiological) doses, it can exacerbate the cytotoxic process, while eliciting cardioprotection at physiological concentrations (48). Accordingly, NO decreases heart rate and myocardial contractility. Mechanistically, these actions include NO binding to mitochondrial proteins with consequent suppression of the electron-transport chain and reduction of mitochondrial energy production. In the presence of regional hypoxia, NO interacts with the mitochondrial permeability transition pore to limit postischemic myocardial damage, hence lowering O_2 demand and reactive oxygen species-dependent damage (24, 81). These effects are reinforced by an NO-cyclic guanosine monophosphate-protein kinase G-mediated limitation of apoptosis (50).

Nitrite is now recognized as a relevant endocrine store of NO and major cardiac modulator (76). It elicits a biphasic protection, as observed in several I/R models, such as the *in vitro* perfused Langendorff rat heart (81) and an *in vivo* murine model of myocardial infarction (22). Since neither S-nitrosothiol nor iron-nitrosyl-protein adducts ameliorates cardiac microvascular perfusion and contractile performance while reducing infarct size and apoptosis, nitrite is suggested as the bioactive intravascular NO-species responsible for such cardioprotection (32). Nitrite-induced cardioprotection is also corroborated by *in vivo* and *in vitro* studies on rat models of myocardial I/R injury that excluded the involvement of nitrate (3). Some mechanisms for nitrite-induced protection were identified on ischemic heart and liver, in which exposure to physiological concentrations of nitrite potently limits cardiac and hepatic reperfusion damages *via* an increased mitochondrial oxidative phosphorylation (68). In particular, nitrite dose dependently blocks complex I by posttranslational S-nitrosation. This reduces electron transfer, decreasing reperfusion-dependent reactive oxygen species production, and ameliorates oxidative inactivation of complexes II - IV and aconitase. Consequently, the opening of mitochondrial permeability transition pore and cytochrome c release is prevented (68). Nitrite-dependent cardioprotection may also involve mitochondrial ubiquinol and cytochrome c oxidase (11), or protein S-nitrosation and iron nitrosylation, or NO-independent processes (8). Accordingly, nitrite, such as NOS-generated NO, may function *via* multiple pathways that coexist within the same cell type. The mechanistic multiplicity of the nitrite-elicited cardioprotection was demonstrated by Perlman *et al.* (65), who demonstrated that brief elevations of plasma nitrite rapidly increase the generation of nitroso- and

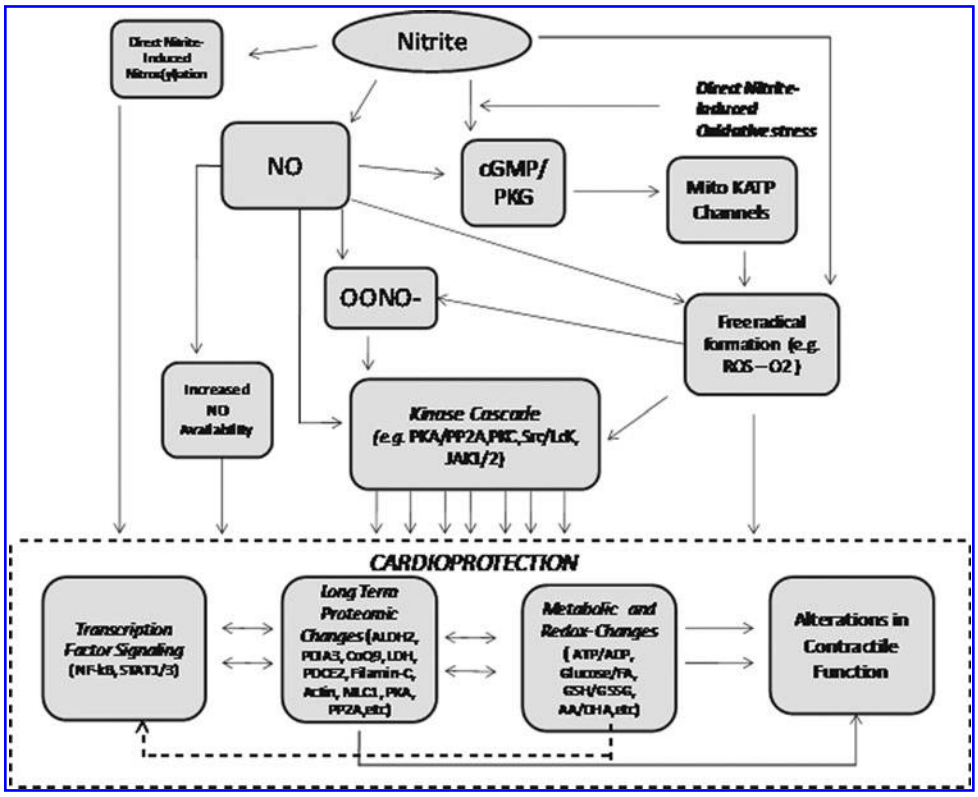

FIG. 4. Mechanisms underlying nitrite-induced cardioprotection (*i.e.*, late preconditioning). AA, ascorbic acid; ALDH2, mitochondrial aldehyde dehydrogenase; CoQ9, ubiquinone biosynthesis protein; DHA, dehydroascorbic acid; FA, formic acid; GSH/GSSG, glutathione oxidation status; JAK1/2, janus kinases; LDH, lactate dehydrogenase; MLC1, myosin light chain protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDC-E2, dehydrolipamide *S*-acetyl transferase; PDIA3, protein disulfide-isomerase A3; PP2A, serine/threonine protein phosphatase 2A; ROS, reactive oxygen species; STAT1/3, signal transducers and activators of transcription. Modified from ref. (76).

nitrosyl species, moderately affect short-term cardiac redox status and long-term oxidative stress, and prolong reductions in glutathione oxidation (oxidated glutathione/reducted glutathione), all these effects being cardioprotective. Due to the differences between cardiac ischemic preconditioning and postconditioning (34), future studies are needed to comparatively evaluate the nitrite-dependent beneficial and/or deleterious effects under both types of I/R conditioning.

Recently, cardiac Mb, in addition to its respiratory function, has been shown to act not only as a potent NO scavenger but also as an allosteric nitrite-reductase enzyme involved in the NO-NO_2^- cycle (66). Under hypoxic and anaerobic conditions, cardiac Mb elicits heme-based reduction of nitrite to NO, which is able to inhibit mitochondrial respiration by NO binding to cytochrome c oxidase, thus modulating O_2 consumption and myocardial energetics (66). Therefore, NO, NO_2^-, and Mb may act in concert to influence the cardiac redox homeostasis with profound effects on both normal and physio-pathological contractility and relaxation. By using wild (Mb+/+) and Mb-knockout (Mb−/−) mice, Hendgen-Cotta *et al.* (35) demonstrated that the nitrite reductase activity of deoxy-Mb protects the myocardium exposed to I/R through bioactive NO production. They observed that in the presence of exogenous nitrite, after 30 min of ischemia, Mb+/+hearts showed increased NO and NO-heme complexes levels that, in contrast, were decreased in the Mb−/−counterparts. Compared with the normal hearts, Mb−/−hearts did not recover from ischemia, exhibiting no evidence for nitrite reductase activity in the infarcted tissue. On the whole, these data are consistent with a cytoprotective role of Mb functioning as a nitrite-reductase and a dioxygenase able to produce and scavenge NO in deoxygenated and oxygenated conditions, respectively (Fig. 5). They also enlarged our view on the link between cardiac redox equilibrium and the control of contractile and relaxing performance, paving the way to launch more research aimed at validating the use of nitrite as a novel cardioprotective agent (16, 19, 24).

Apart from facing hypoxia and acidosis, many freshwater fish and amphibians can be exposed to high environmental nitrite concentrations. Plasma nitrite levels in freshwater fish may be above those of the ambient water (4), reaching millimolar range in nitrite-contaminated environments. At these concentrations, nitrite is toxic and can influence ion, respiratory, and circulatory homeostasis of the animal (44). Conceivably, these organisms can provide a plethora of suitable models to explore whether the Mb-NO-nitrite circuit contributes to keep in balance intracardiac redox equilibrium and elevated myocardial resistance to O_2 deprivation. Further, several Anurans, such as the Hb+/Mb− newt and the Hb+/Mb− Western clawed frog *Xenopus tropicalis* (26, 81, 82), namely the icefish, are natural knock-outs for Hb and Mb and are, thus, well suited for revealing evolutionary and mechanistic aspects of the NO-nitrite system.

Jensen (43) showed in zebrafish that exposure to high environmental nitrite levels, with the consequent accumulation of this anion across the gills, is paralleled by elevated blood levels of nitrosyl-Hb, hence of NO formation *via* an extensive deoxyHb-mediated reduction of nitrite. Canty and Driedzic (9) demonstrated in a teleost fish (the sculpin) that Mb is essential to maintain heart performance under severe hypoxia. More recently, other studies in fish and anuran species provided evidence for an intracardiac NO generation *via* both NOS- and nitrite-dependent mechanisms (1, 12, 28) that contribute to contractile regulation under both basal conditions and physical (*i.e.*, Starling response) and chemical stimulation (*i.e.*, Ach, AngII) (41). Intracardiac NOS expression in the

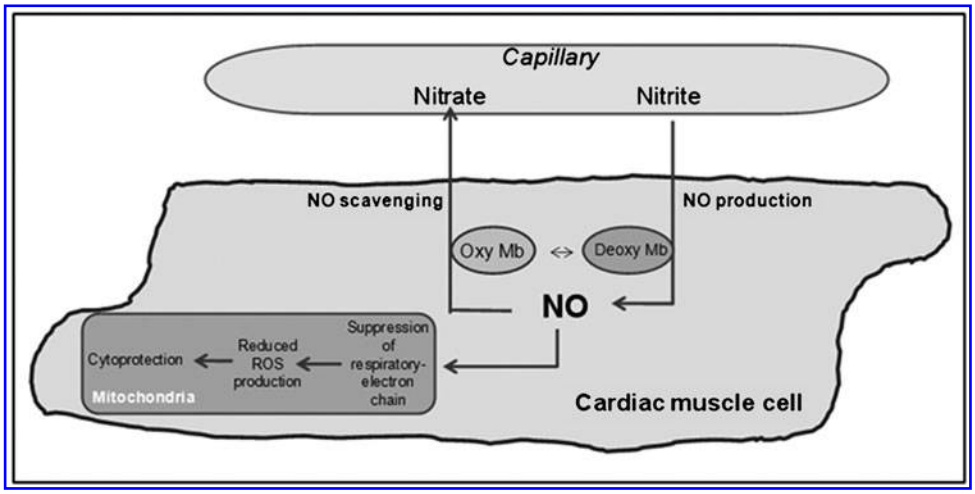

FIG. 5. The nitrite-Mb-NO circuit. The role of Mb in producing and scavenging NO in deoxygenated and oxygenated conditions, respectively, is illustrated. Mb, myoglobin; NO, nitric oxide. Modified from ref. (76).

Antarctic Hb−/Mb− icefish *C. aceratus* is lower than that of its Mb+ counterpart, Hb−/Mb+ *C. hamatus* (1). *C. aceratus* heart, compared with that of the Mb expressing *C. hamatus*, appears much more sensitive to NOS stimulation by the authentic substrate L-arginine (12). Since cardiac Mb nitrite reductase activity is absent and NOS is poorly expressed, other mechanisms may contribute to local NO production under these conditions. It is possible that, in absence of the Mb-mediated scavenging effect, the NO half-life is increased (larger free NO availability), compensating for the reduced NOS expression. Contrarily, under Hb null but Mb expressing conditions, that is, in *C. hamatus*, cardiac Mb may contribute to local NO generation by reducing nitrite, thus maintaining nitrergic and redox homeostasis. In fact, in this fish, very low concentrations of exogenous nitrite (0.1 μmol/L) induce a positive inotropic effect which is similar to that elicited by NO (12). At the same time, as suggested in mammals (79), the well-expressed NOS system might represent a notable source of NO from added nitrite. Noteworthy, it was suggested that the high NO levels occurring in the absence of both Hb and Mb have promoted in the icefish some of the major cardiovascular and subcellular compensations mentioned above. The latter include mitochondrial enlargement in the myocardiocytes (77), which contributes to myocardial oxidative equilibrium and, hence, heart protection from the risk of hypoxemic hypoxia.

The correlation between nitrite-NO equilibrium, O_2 utilization, and heart performance has been studied in ventricular preparations of the anoxia tolerant turtle *T. scripta* (55). It was shown that NOS-dependent NO production is accompanied by reduced O_2 consumption and increased contractility, without affecting developed force and lactate production. Since this effect is stronger in hypoxia, it is conceivable that NO, in spite of the limited O_2 availability, preserves cardiac function by depressing mitochondrial respiration rate while improving contractile efficiency. This may contribute to the notable anoxia resistance of the turtle heart.

Emerging Evidences of Hydrogen Sulphide-Induced Tolerance to Hypoxia

Recently, a new member joined the family of the biological active gases (the so-called gasotransmitters): the free diffusible H_2S. It shows biochemical properties similar to carbon monoxide and NO (80). It is toxic at high concentration, because of the inhibition of mitochondrial respiration chain at the level of cytochrome oxidase *c*, which causes metabolic hypoxia (Fig. 6A). However, akin to NO and CO, H_2S is now recognized to be involved in several physiological processes such as long term synaptic potentiation, cardioprotection, and vasodilation (53). On an evolutionary point of view, elevated levels of H_2S with concomitant hypoxia have been associated to massive extinctions promoting survival of animals that developed protection against elevated levels of toxic gases. It is noteworthy that competition between O_2 and H_2S has a long evolutionary history and they have been, in turn, considered "pollutant" because of the rapid increase of their partial pressure in the atmosphere. Before photosynthetic organisms produced enough O_2 to saturate the atmosphere, the terminal acceptor of electron in the respiratory chain is believed to be a sulfur atom. Therefore, it is not surprising that sulfide can be rapidly internalized by cells and react with intracellular compounds taking part in the basic biochemical process in cells. Moreover, in several species, the balance between the two gases seems to be critical in the regulation of pulmonary vasoconstriction (62). Interestingly, in vertebrates, H_2S is produced at low, but detectable, levels by at least three constitutively expressed enzymes: by cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (Fig. 6B). Until recently, H_2S was considered only a side product in the activity of these enzymes, but this postulate was proved wrong. In fact, as demonstrated by CSE knock-out mice, a basal production of H_2S is necessary to maintain physiological functions (84).

Aside from the endogenous H_2S, it has also been demonstrated that administration of H_2S donors may be useful to reduce ischemia/reperfusion injuries in the rat heart (13). At first, Geng and coworkers demonstrated that inhibition of CSE resulted in a increased isoproterenol-induced myocardial injury showing the pivotal role of endogenous H_2S in the attenuation of myocytes injury (29). The anti-apoptotic and protective action of exogenous H_2S was consistently effective in a model of regional ischemia/reperfusion (72). In the attempt of elucidating the mechanisms of action selective blockers of the K_{ATP}, sensitive channels were shown to play a pivotal role in the signal cascade leading to protection (47). The preconditioning-like effect induced by exogenous H_2S is mediated by protein kinase C, and the cardioprotection

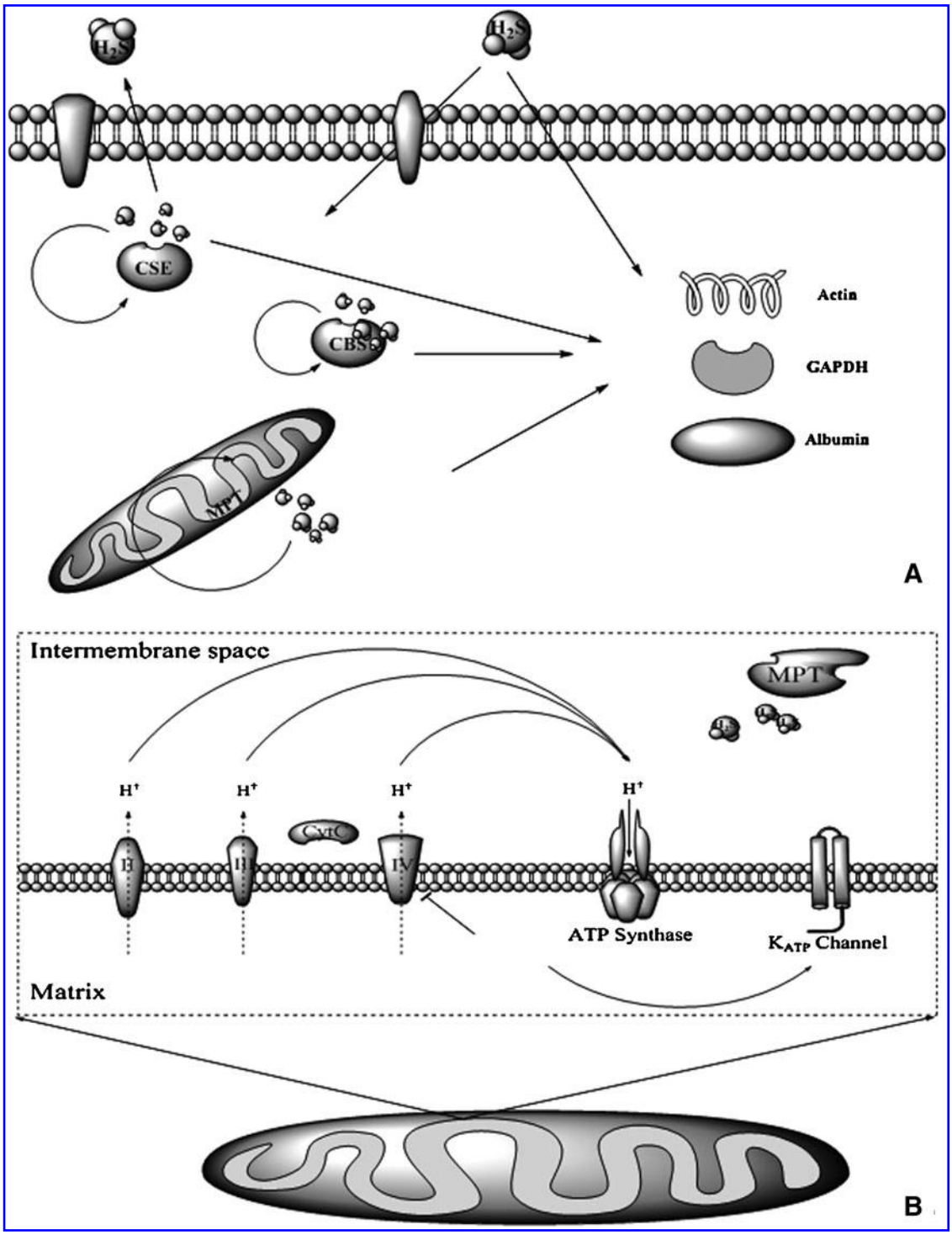

FIG. 6. Intracellular processes activated by H_2S. (**A**) Main sources of endogenous H_2S: CSE, CBS, MPT. Both endogenously produced and exogenous H_2S can freely diffuse through the lipid bilayer and without facilitated transport. Intracellular H_2S targets several proteins including H_2S-producing enzymes (CBS and CSE), cytoskeletal proteins (actin), and free proteins (albumin). In mitochondria, H_2S activates K_{ATP} channels. (**B**) Proposed mechanism of H_2S toxicity: H_2S, such as hydrogen cyanide, blocks the electron transport chain at the level of cytochrome C oxidase (IV) inhibiting respiration. In mitochondria, H_2S is produced by MPT and leads to the opening of mitochondrial K_{ATP} channels. CBS, cystathionine beta-synthase; CSE, cystathionine gamma-lyase; H_2S, hydrogen sulfide; K_{ATP}, ATP-sensitive potassium; MPT, 3-mercaptopyruvate sulfurtransferase.

induced by sodium hydroxide has been shown to have a dose-response relation with the most beneficial outcome at 1 μmol/kg (63). The putative effect of the gas during the reperfusion phase is under debate. In fact, although some authors demonstrated the possibility to simulate ischemic postconditioning by administration of H_2S donors during reperfusion in isolated heart models (46), some doubts arise from the possible exacerbation of inflammatory processes *in vivo* models.

Interestingly, constitutively produced H_2S has been proposed as a pivotal mediator in the O_2 sensing mechanisms of vertebrates (60). In fact, exogenous addiction of cysteine, the substrate for CBS and CSE, increases the vasoconstriction induced by hypoxia in lamprey, rat, and bovine (60). A similar effect was shown in trout gills, confirming that H_2S and hypoxia act through a similar mechanism, also indicating a relation between endogenous H_2S and the O_2 sensing process (61). A role for H_2S as an O_2 sensor was suggested by the observation that both endogenous and exogenous H_2S reduces hypoxia/reoxygenation injuries in several experimental setups. Particular attention has been paid to the effect of H_2S on myocardial ischemia/reperfusion injuries. Administration of H_2S donors either before or after an infracting ischemia reduces infarct size and improves postischemic left ventricular recovery. Therefore, H_2S can be considered both a preconditioning and a postconditioning agent for myocardial protection. Notably, the beneficial effects of H_2S treatment before a potentially lethal ischemia are not exclusive of the myocardium. In fact, administration of exogenous is cytoprotective in a model of experimentally induced ischemia in the rat liver (45). Similarly, in a model of lungs subjected to ischemia/reperfusion, beneficial effects are elicited by H_2S donors, whereas a more severe injury is elicited by CBS inhibition (25). Analogously, renal function is recovered by exogenous H_2S administration when endogenous production is blunted by inhibiting CBS (83). It is also now accepted that H_2S exerts potent antioxidant effects and can upregulate antioxidant defenses under more chronic conditions (49).

The Phenomenon of H_2S-Induced Hibernation (Suspended Animation)

Competition between O_2 and H_2S has a long evolutionary history and they have been, in turn, considered "pollutant." Before photosynthetic organisms produced enough O_2 to saturate the atmosphere, the terminal acceptor of electron in the respiratory chain is believed to be a sulfur atom. Therefore, it is not surprising that sulfide can be rapidly internalized by cells and react with intracellular compounds taking part to the basic cellular biochemical process. One of the most attracting features of H_2S is the ability to induce a suspended animation in nonhibernating species. In fact, a hibernated-like state has been obtained in mice with the use of inhaled H_2S, resulting in a dramatic reduction of the MR (6). This state is characterized by a $\sim$50% reduction in O_2 consumption and a $\sim$60% drop in carbon dioxide output. However, when the animals were returned to atmospheric conditions, normal functions were restored with no macroscopic defects. These effects are consistent with the observation that inhibition of the respiratory chain causes a drastic reduction of MR in several organisms (58). Being a selective, although reversible, inhibitor of cytochrome oxidase c, H_2S mimics hypoxic conditions, and the observed effects are very similar to the initial phase of hibernation (10). The slowing of respiration through the competition with O_2 for binding cytochrome oxidase c has been also demonstrated with synthetic models of the enzyme (15). The possibility to induce a reversible hibernation at will with H_2S has been proved effective also in a rat model in which preexposure to the gas reduced body temperature and gastric ulceration after immersion and restraint stress (51).

Conclusions and Perspectives

One of the major challenges of cardiovascular reserchers is to understand the molecular and cellular mechanisms underlying heart vulnerability *vs.* resistance under conditions of O_2 deprivation. Studies based on postgenomic techniques provide powerful insights into the mechanisms underpinning the hypoxic stress response, identifying a large number of candidate gene targets. Therefore, hypoxia-relevant phenotypes can now be related to gene function, which sustain tissue-specific pattern of resistance. In this context, comparative studies on hypoxia-tolerant vertebrate species, as well as on evolutionary *vs.* artificial mutant models, are likely to hold important clues for revealing fundamental mechanisms of cardiovascular tolerance and how they have evolved. From a heuristic point of view, such a comparative approach will also suggest these natural heart models as powerful tools that complement the more traditional models of human diseases, such as the mouse, thus improving human health.

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References

1. Amelio D, Garofalo F, Pellegrino D, Giordano F, Tota B, and Cerra MC. Cardiac expression and distribution of nitric oxide synthases in the ventricle of the cold-adapted Antarctic teleosts, the hemoglobinless *Chionodraco hamatus* and the red-blooded Trematomus bernacchii. *Nitric Oxide* 15: 190–198, 2006.
2. Bailey JR, Rodnick KJ, MacDougall R, Clowe S, and Driedzic WR. Anoxic performance of the American eel (*Anguilla rostrata L.*) heart requires extracellular glucose. *J Exp Zool* 286: 699–706, 2000.
3. Baker JE, Su J, Fu X, Hsu A, Gross GJ, Tweddell JS, and Hogg N. Nitrite confers protection against myocardial infarction: role of xanthine oxidoreductase, NADPH oxidase and K(ATP) channels. *J Mol Cell Cardiol* 43: 437–444, 2007.
4. Bath RN and Eddy FB. Transport of nitrite across fish gills. J Exp Zool 214: 119–121, 1980.
5. Bickler PE and Buck LT. Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. *Annu Rev Physiol* 69: 5.1–5.26, 2007.
6. Blackstone E, Morrison M, and Roth MB. H_2S induces a suspended animation-like state in mice. *Science* 308: 518, 2005.
7. Broom DM and Johnson KG. *Stress and Animal Welfare*. London: Chapman & Hall, 1993.
8. Bryan NS, Fernandez BO, Bauer SM, Garcia-Saura MF, Milsom AB, Rassaf T, Maloney RE, Bharti A, Rodriguez J, and Feelisch M. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat Chem Biol* 1: 290–297, 2005.

9. Canty AA and Driedzic WR. Evidence that myoglobin does not support heart performance at maximal levels of oxygen demand. *J Exp Biol* 128: 469–473, 1987.
10. Carey HV, Andrews MT, and Martin SL. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 83: 1153–1181, 2003.
11. Castello PR, David PS, McClure T, Crook Z, and Poyton RO. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab* 3: 277–287, 2006.
12. Cerra MC, Angelone T, Parisella ML, Pellegrino D, and Tota B. Nitrite modulates contractility of teleost (*Anguilla anguilla* and *Chionodraco hamatus*, i.e., the Antarctic hemoglobinless icefish) and frog (*Rana esculenta*) hearts. *Biochim Biophys Acta* 1787: 849–855, 2009.
13. Chang L, Geng B, Yu F, Zhao J, Jiang H, Du J, and Tang C. Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. *Amino Acids* 34: 573–585, 2008.
14. Chen J, Zhu JX, Wilson I, and Cameron JS. Cardioprotective effects of K_{ATP} channel activation during hypoxia in goldfish *Carassius auratus*. *J Exp Biol* 208: 2765–2772, 2005.
15. Collman JP, Ghosh S, Dey A, and Decreau RA. Using a functional enzyme model to understand the chemistry behind hydrogen sulfide induced hibernation. *Proc Natl Acad Sci USA* 106: 22090–22095, 2009.
16. Cossins A and Berenbrink M. Physiology: myoglobin's new clothes. *Nature* 454: 416–417, 2008.
17. Cossins AR and Bowler K. *Temperature Biology of Animals*. London: Chapman & Hall, 1987.
18. Cossins A, Fraser J, Hughes M, and Gracey A. Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *J Exp Biol* 209: 2328–2336, 2006.
19. Crowder WC, Nie M, and Ultsch GR. Oxygen uptake in bullfrog tadpoles (*Rana catesbeiana*). *J Exp Zool* 280: 121–134, 1998.
20. Di Maio A and Block BA. Ultrastructure of the sarcoplasmic reticulum in cardiac myocytes from Pacific bluefin tuna. *Cell Tiss Res* 334: 121–134, 2008.
21. Driedzic WR and Gesser H. Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis, and low temperature. *Physiol Rev* 74: 221–258, 1994.
22. Duranski MR, Greer JJ, Dejam A, Jaganmohan S, Hogg, N, Langston W, Patel RP, Yet SF, Wang X, Kevil CG, Gladwin MT, and Lefer DJ. Cytoprotective effects of nitrite during *in vivo* ischemia-reperfusion of the heart and liver. *J Clin Invest* 115: 1232–1240, 2005.
23. Farrell AP and Stecyk JAW. The heart as a working model to explore themes and strategies for anoxic survival in ectothermic vertebrates. *Comp Biochem Physiol A* 147: 300–312, 2007.
24. Flögel U, Fago A, and Rassaf T. Keeping the heart in balance: the functional interactions of myoglobin with nitrogen oxides. *J Exp Biol* 213 (Pt 16): 2726–2733, 2010.
25. Fu Z, Liu X, Geng B, Fang L, and Tang C. Hydrogen sulfide protects rat lung from ischemia-reperfusion injury. *Life Sci* 82: 1196–1202, 2008.
26. Fuchs C, Hankeln T, and Burmester T. The amphibian globin gene repertoire as revealed by the Xenopus genome. *Cytogenet Genome Res* 112: 296–306, 2006.
27. Garofalo F, Pellegrino D, Amelio D, and Tota B. The Antarctic hemoglobinless icefish, fifty five years later: a unique cardiocirculatory interplay of disaptation and phenotypic plasticity. *Comp Biochem Physiol A Mol Integr Physiol* 154: 10–28, 2009.
28. Gattuso A, Mazza R, Pellegrino D, and Tota B. Endocardial endothelium mediates luminal ACh–NO signaling in the isolated frog heart. *Am J Physiol* 276: H633–H641, 1999.
29. Gemelli L, Martino G, and Tota B. Oxidation of lactate in the compact and spongy myocardium of tuna fish (*Thunnus thynnus thynnus* L.). *Comp Biochem Physiol B* 65: 321–326, 1980.
30. Geng B, Chang L, Pan C, Qi Y, Zhao J, Pang Y, Du J, and Tang C. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. *Biochem Biophys Res Commun* 318: 756–763, 2004.
31. Gesser H and Poupa O. Acidosis and cardiac muscle contractility: comparative aspects. *Comp Biochem Physiol* 76: 559–566, 1983.
32. Gonzales RJ, Bryant JM, Naik JS, Resta TC, and Walker BR. The ubiquitous role of nitric oxide in cardioprotection. *Microcirculation* 15: 473–483, 2008.
33. Hanley PJ and Daut J. K(ATP) channels and preconditioning: a re-examination of the role of mitochondrial K(ATP) channels and an overview of alternative mechanisms. *J Mol Cell Cardiol* 39: 17–50, 2005.
34. Hausenloy DJ, Wynne AM, and Yellon DM. Ischemic preconditioning targets the reperfusion phase. *Basic Res Cardiol* 102: 445–452, 2007.
35. Hendgen-Cotta UB, Merx MW, Shiva S, Schmitz J, Becher S, Klare JP, Steinhoff HJ, Goedecke A, Schrader J, Gladwin MT, Kelm M, and Rassaf T. Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 105: 10256–10261, 2008.
36. Herbert CV and Jackson DC. Temperature effects on the responses to prolonged submergence in the turtle Chrysemys picta bellii. II. Metabolic rate, blood acid-base and ionic changes and cardiovascular function in aerated and anoxic water. *Physiol Zool* 58: 670–681, 1985.
37. Hicks JMT and Farrell AP. The cardiovascular responses of the red-eared slider (*Trachemys scripta*) acclimated to either 22 or 5°C. I. Effects of anoxia exposure on *in vivo* cardiac performance. *J Exp Biol* 203: 3765–3774, 2000.
38. Hochachka PW. *Living Without Oxygen*. Cambridge: Harvard University Press, 1980.
39. Hochachka PW. Defense strategies against hypoxia and hypothermia. *Science* 231: 234–241, 1986.
40. Hochachka PW and Somero GN. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford University Press, 2002.
41. Imbrogno S, Cerra MC, and Tota B. Angiotensin II-induced inotropism requires an endocardial endothelium-nitric oxide mechanism in the *in-vitro* heart of *Anguilla anguilla*. *J Exp Biol* 206: 2675–2684, 2003.
42. Jackson DC. Cardiovascular function in turtles during anoxia and acidosis: *In vivo* and *in vitro* studies. *Am Zool* 27: 49–56, 1987.
43. Jensen FB. Nitric oxide formation from nitrite in zebrafish. *J Exp Biol* 210: 3387–3394, 2007.
44. Jensen FB. The role of nitrite in nitric oxide homeostasis: a comparative perspective. *Biochim Biophys Acta* 1787: 841–848, 2009.
45. Jha S, Calvert JW, Duranski MR, Ramachandran A, and Lefer DJ. Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. *Am J Physiol Heart Circ Physiol* 295: 801–806, 2008.

46. Ji Y, Pang QF, Xu G, Wang L, Wang JK, and Zeng YM. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur J Pharmacol* 587: 1–7, 2008.
47. Johansen D, Ytrehus K, and Baxter GF. Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury—evidence for a role of K ATP channels. *Basic Res Cardiol* 101: 53–60, 2006.
48. Jones SP and Bolli R. The ubiquitous role of nitric oxide in cardioprotection. *J Mol Cell Cardiol* 40: 16–23, 2006.
49. Kimura Y and Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 18: 1165–1167, 2004.
50. Korge P, Honda HM, and Weiss JN. Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. *Proc Natl Acad Sci USA* 99: 3312–3317, 2002.
51. Lou LX, Geng B, Du JB, and Tang CS. Hydrogen sulphide-induced hypothermia attenuates stress-related ulceration in rats. *Clin Exp Pharmacol Physiol* 35: 223–228, 2008.
52. Lutz PL and Nilsson GE. Vertebrate brains at the pilot light. *Respir Physiol Neurobiol* 141: 285–296, 2004.
53. Mancardi D, Penna C, Merlino A, Del Soldato P, Wink DA, and Pagliaro P. Physiological and pharmacological features of the novel gasotransmitter: hydrogen sulfide. *Biochim Biophys Acta* 1787: 864–872, 2009.
54. Mathur GB. Anaerobic respiration in a Cyprinoid fish *Rasbora daniconius* (Ham). *Nature* 214: 318–319, 1967.
55. Misfeldt M, Fago A, and Gesser H. Nitric oxide increases myocardial efficiency in the hypoxia-tolerant turtle *Trachemys scripta*. *J Exp Biol* 212: 954–960, 2009.
56. Nilsson GE. Surviving anoxia with the brain turned on. *News Physiol Sci* 16: 218–221, 2001.
57. Nilsson GE, Rosen P, and Johannson D. Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. *J Exp Biol* 180: 153–162, 2003.
58. Nystul TG and Roth MB. Carbon monoxide-induced suspended animation protects against hypoxic damage in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 101: 9133–9136, 2004.
59. O'Brien KM and Sidell BD. The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *J Exp Biol* 203: 1287–1297, 2000.
60. Olson KR, Dombkowski RA, Russell MJ, Doellman MM, Head SK, Whitfield NL, and Madden JA. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J Exp Biol* 209: 4011–4023, 2006.
61. Olson KR, Healy MJ, Qin Z, Skovgaard N, Vulesevic B, Duff DW, Whitfield NL, Yang G, Wang R, and Perry SF. Hydrogen sulfide as an oxygen sensor in trout gill chemoreceptors. *Am J Physiol Regul Integr Comp Physiol* 295: 669–680, 2008.
62. Olson KR, Whitfield NL, Bearden SE, St. Leger J, Nilson E, Gao Y, and Madden JA. Hypoxic pulmonary vasodilation: a paradigm shift with a hydrogen sulfide mechanism. *Am J Physiol Regul Integr Comp Physiol* 298: R51–R60, 2009.
63. Padilla PA and Roth MB. Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc Natl Acad Sci USA* 98: 7331–7335, 2001.
64. Pan TT, Chen YQ, and Bian JS. All in the timing: a comparison between the cardioprotection induced by H_2S preconditioning and post-infarction treatment. *Eur J Pharmacol* 616: 160–165, 2009.
65. Perlman DH, Bauer SM, Ashrafian H, Bryan NS, Garcia-Saura MF, Lim CC, Fernandez BO, Infusini G, McComb ME, Costello CE, and Feelisch M. Mechanistic insights into nitrite-induced cardioprotection using an integrated metabolomic/proteomic approach. *Circ Res* 104: 796–804, 2009.
66. Rassaf T, Flögel U, Drexhage C, Hendgen-Cotta U, Kelm M, and Schrader J. Nitrite reductase function of deoxymyoglobin: oxygen sensor and regulator of cardiac energetics and function. *Circ Res* 100: 1749–1754, 2007.
67. Schjolden J, Stoskhus A, and Winberg S. Does individual variation in stress responses and agonistic behavior reflect divergent stress coping strategies in juvenile rainbow trout? *Physiol Biochem Zool* 78: 715–723, 2005.
68. Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, and Gladwin MT. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med* 204: 2089–2102, 2007.
69. Shoubridge EA and Hochachka PW. Ethanol: novel end product of vertebrate anaerobic metabolism. *Science* 209: 308–309, 1980.
70. Sidell BD. Intracellular oxygen diffusion: the roles of myoglobin and lipid at cold body temperature. *J Exp Biol* 201 (Pt 8): 1119–1128, 1998.
71. Sidell BD and O'Brien KM. When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *J Exp Biol* 209: 1791–1802, 2006.
72. Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, Cuzzocrea S, Fantozzi R, and Thiemermann C. Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. *Shock* 31: 267–274, 2009.
73. Stecyk JA and Farrell AP. Effects of extracellular changes on spontaneous heart rate of normoxia- and anoxia-acclimated turtles (*Trachemys scripta*). *J Exp Biol* 210: 421–431, 2007.
74. Stecyk JAW, Paajanen V, Farrell AP, and Vornanen M. Effect of temperature and prolonged anoxia exposure on electrophysiological properties of the turtle (*Trachemys scripta*) heart. *Am J Physiol* 293: 421–437, 2007.
75. Stecyk JAW, Stenløcken KO, Farrell AP, and Nilsson GE. Maintained cardiac pumping in anoxic crucian carp. *Science* 306: 77, 2004.
76. Tota B, Quintieri AM, and Angelone T. The emerging role of nitrite as an endogenous modulator and therapeutic agent of cardiovascular function. *Curr Med Chem* 17: 1915–1925, 2010.
77. Urschel M and O'Brien KM. High mitochondrial densities in the hearts of Antarctic icefishes are maintained by an increase in mitochondrial size rather than mitochondrial biogenesis. *J Exp Biol* 211: 2638–2646, 2008.
78. Van Raaij M, Van den Thillart G, Vianen GJ, Pit DS, Balm PH, and Steffens AB. Substrate mobilization and hormonal changes in rainbow trout (*Oncorhynchus mykiss*, *L.*) and common carp (*Cyprinus carpio*, *L.*) during deep hypoxia and subsequent recovery. *J Comp Physiol* 166: 443–452, 1996.
79. Vanin AF, Bevers LM, Slama-Schwok A, and van Faassen EE. Nitric oxide synthase reduces nitrite to NO under anoxia. *Cell Mol Life Sci* 64: 96–103, 2007.
80. Wang R. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798, 2002.

81. Webb A, Bond R, McLean P, Uppal R, Benjamin N, and Ahluwalia A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia–reperfusion damage. *Proc Natl Acad Sci USA* 101: 13683–13688, 2004.
82. Xi Y, Obara M, Ishida Y, Ikeda S, and Yoshizato K. Gene expression and tissue distribution of cytoglobin and myoglobin in the Amphibia and Reptilia: possible compensation of myoglobin with cytoglobin in skeletal muscle cells of anurans that lack the myoglobin gene. *Gene* 398: 94–102, 2007.
83. Xu Z, Prathapasinghe G, Wu N, Hwang SY, Siow YL, and O K. Ischemia-reperfusion reduces cystathionine-beta-synthase-mediated hydrogen sulfide generation in the kidney. *Am J Physiol Renal Physiol* 297: 27–35, 2009.
84. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R. H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587–590, 2008.

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Abbreviations Used

AA = ascorbic acid
ACh = acetylcholine
ALDH2 = mitochondrial aldehyde dehydrogenase
Ang II = angiotensin II
ATP = adenosine triphosphate
CBS = cystathionine beta-synthase
CO = carbonic oxide
CoQ9 = ubiquinone biosynthesis protein
CSE = cystathionine gamma-lyase
DHA = dehydroascorbic acid
eNOS = endothelial nitric oxide synthase
FA = formic acid
GSH = reducted glutathione
GSSG = oxidated glutathione
H_2S = hydrogen sulfide
Hb = haemoglobin
HIF-1 = hypoxia-inducible factor-1
HR = heart rate
I/R = ischemic/reperfusion
JAK1/2 = janus kinase
K_{ATP} = ATP-sensitive potassium
LDH = lactate dehydrogenase
Mb = myoglobin
MLC1 = myosin light chain 1
MPT = 3-mercaptopyruvate sulfurtransferase
NADP = nicotidamide adenine dinucleotide phosphate
NADPH = nicotinamide-adenine dinucleotide (phosphate) dehydrogenase
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
NO = nitric oxide
NO_2^- = nitrite
NOS = NO synthases
O_2 = oxygen
PDC-E2 = dehydrolipamide S-acetyl transferase
PDIA3 = protein disulfide-isomerase A3
PP2A = serine/threonine protein phosphatases2A
ROS = reactive oxygen species
SRC/LCK = proto-oncogenic SRC protein tyrosine kinase
STAT1/3 = signal transducer and activator of transcription 3
SV = stroke volume
VEGF = vascular endothelial growth factor

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3. Pasquale Pagliaro , Francesca Moro , Francesca Tullio , Maria-Giulia Perrelli , Claudia Penna . 2011. Cardioprotective Pathways During Reperfusion: Focus on Redox Signaling and Other Modalities of Cell Signaling. *Antioxidants & Redox Signaling* **14**:5, 833-850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
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