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Commentary

Hydrogen sulfide and oxygen sensing: implications in cardiorespiratory control

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Summary

Although all cells are variously affected by oxygen, a few have the responsibility of monitoring oxygen tensions and initiating key homeostatic responses when P_{O_2} falls to critical levels. These 'oxygen-sensing' cells include the chemoreceptors in the gills (neuroepithelial cells), airways (neuroepithelial bodies) and vasculature (carotid bodies) that initiate cardiorespiratory reflexes, oxygen sensitive chromaffin cells associated with systemic veins or adrenal glands that regulate the rate of catecholamine secretion, and vascular smooth muscle cells capable of increasing blood flow to systemic tissues, or decreasing it through the lungs. In spite of intense research, and enormous clinical applicability, there is little, if any, consensus regarding the mechanism of how these cells sense oxygen and transduce this into the appropriate physiological response. We have recently proposed that the metabolism of hydrogen sulfide (H_2S) may serve as an 'oxygen sensor' in vertebrate vascular smooth muscle and preliminary evidence suggests it has similar activity in gill chemoreceptors. In this proposed mechanism, the cellular concentration of H_2S is determined by the simple balance between constitutive H_2S production in the cytoplasm and H_2S oxidation in the mitochondria; when tissue oxygen levels fall the rate of H_2S oxidation decreases and the concentration of biologically active H_2S in the tissue increases. This commentary briefly describes the oxygen-sensitive tissues in fish and mammals, delineates the current hypotheses of oxygen sensing by these tissues, and then critically evaluates the evidence for H_2S metabolism in oxygen sensing.

Key words: chemoreceptors, carotid body, neuroepithelial cells.

Introduction

Hypoxia of external or internal origin is a potential and recurring threat to all cells. Vertebrates have developed complex and specialized oxygen-sensing tissues to monitor environmental hypoxia, blood hypoxia (hypoxemia) and even local tissue hypoxia. Numerous theories have been advanced to explain how these cells monitor oxygen (O_2) levels and how low oxygen signals are transduced into physiological responses. However, no single theory has emerged to date to unequivocally explain the O_2-sensing signal transduction process in any of these tissues. Hydrogen sulfide (H_2S) has recently emerged as a signaling 'gasotransmitter' of considerable biological potential and we have proposed that O_2-dependent metabolism of H_2S may be the 'O_2 sensor' in both vascular and non-vascular smooth muscle as well as putative chemoreceptor cells, the neuroepithelial cells. This commentary identifies current theories and concepts of O_2 sensing during acute hypoxia in fish and mammals, vertebrates at opposite poles of oxygen availability and demand, and provides evidence for H_2S metabolism in the O_2-sensing mechanism. Some of the remaining questions and problems associated with this hypothesis, and of H_2S as a signaling molecule in general, are also considered.

Types of hypoxia and importance of oxygen sensing

Tissue hypoxia can have an external or internal origin. External (ambient) hypoxia occurs in aquatic environments seasonally, diurnally or episodically, and is a common feature of both freshwater and saltwater environments (Bickler and Buck, 2007). Compared to air, aquatic hypoxia is especially problematic because water has a lower capacity for O_2 (1/30), diffusion through water is slower (Krogh's diffusion coefficients 1/200000) and O_2 solubility decreases by around 20% for every 10°C increase in temperature and another 20% in seawater compared to freshwater. In addition, the high viscosity of water (60 times that of air) impedes breathing, and even mixing of O_2. Life on land has problems of its own. Terrestrial animals may be exposed to hypoxia when they ascend to higher altitudes due to the fall in barometric pressure (at 3000 m P_{O_2} is 30% lower than at sea level) or enter burrows where there may be little convective delivery of fresh air (Ramirez et al., 2007). Even the mammalian fetus experiences a form of external hypoxia. Global internal hypoxia can originate from a decrease in the ventilation/perfusion ratio in the respiratory organs resulting in a decrease in O_2 uptake, or it can be more localized following an increase in the metabolism/perfusion ratio in systemic tissues. Low O_2 content in blood is called hypoxemia and if it produces tissue hypoxia this is hypoxemic hypoxia. Ischemia (or ischemic hypoxia) refers to tissue hypoxia resulting from insufficient blood flow.

In order to adequately and rapidly respond to potential shortfalls in oxygen, animals have developed O_2-sensing tissues capable of detecting hypoxia in the environment, in blood, or within tissues. Notable among these are chemoreceptors that initiate central cardio-respiratory responses, chromaffin cells in the adrenal medulla or central veins that provide a humoral source of catecholamines and blood vessels that exert local control over tissue perfusion (Lahiri et al., 2006; Milsom and Burleson, 2007; Reid and Perry, 2003; Weir et al., 2005). While all cells are affected by hypoxia, these 'O_2-sensing' cells appear exquisitely sensitive to subtle degrees of hypoxia and thereby serve important homeostatic functions during the initial (acute) hypoxic challenge. Long-term (chronic) effectors of hypoxic responses that regulate genetic

responses, such as the hypoxia inducible factor (HIF) transcription factor family, sustain and augment the initial responses but are not considered here.

Oxygen-sensing tissues

Monitors of external hypoxia

Tissues: gill and airway neuroepithelial cells

Fish and other aquatic breathing vertebrates such as most larval and a few adult amphibians (e.g. tadpoles, frogs and salamanders and caecilians) are for reasons described above the most susceptible to environmental hypoxia. Individual neuroepithelial cells (NECs) are scattered over the gill surface, but are especially prevalent on the leading (inhalant) and distal edge of the gill filament. In hypoxia-sensitive fish such as trout, they initiate a reflex bradycardia and increase ventilatory rate and amplitude when water P_{O_2} falls to 75–100 mmHg (Reid and Perry, 2003) (for a review, see Gilmour and Perry, 2007). In some fish (e.g. trout) these NECs are predominantly on the first gill arch, the homolog of the mammalian carotid body; in others (bowfin, catfish) they are more evenly distributed over the gills and even the orobranchial cavity (Gilmour and Perry, 2007; Milsom and Burleson, 2007). These externally oriented receptors are absent in the obligate air-breathing South American lungfish, *Lepidosiren paradoxa*, and it has been suggested that this mode of O_2 sensing was lost early in vertebrate evolution (Sanchez et al., 2001).

Cells similar to NECs are grouped in clusters called neuroepithelial bodies (NEBs) that line the airways of mammalian lungs especially near bifurcations (Peers and Kemp, 2001; Kemp et al., 2002). NEBs are most prominent in neonatal lungs and are believed to monitor airway P_{O_2} and help initiate breathing and thereafter optimize ventilation/perfusion matching through central reflexes and by paracrine stimulation of adjacent pulmonary vasculature (Kemp et al., 2002). Although they are more responsive to airway hypoxia than hypoxemia, their function as general monitors of environmental P_{O_2} in adult animals is unclear.

Monitors of blood hypoxemia

Tissues: gill, carotid, chromaffin/adrenal

Monitors of blood O_2 status such as vascularly oriented NECs in fish gills and carotid bodies in tetrapods provide general information on O_2 delivery to systemic tissues and, not unexpectedly, are predominantly associated with the arterial circulation. In addition, O_2-sensitive chromaffin cells are variously found in heart and vasculature of the more ancient vertebrates and condensed in the adrenal medulla of mammals. Their location suggests that they provide information on the balance between O_2 delivery and tissue utilization.

Gill NECs

Vascularly oriented NECs are found on all gill arches and in some fish are especially prevalent surrounding the efferent branchial artery, the main vessel draining oxygenated blood from the gill (Milsom and Burleson, 2007). Here, they are ideally situated to monitor blood oxygenation and, unlike mammals, they may better distinguish blood O_2 content than blood P_{O_2}. Like other gill NECs and cells of the NEB they secrete serotonin in response to hypoxemia.

Carotid bodies

The carotid bodies of mammals are small organelles located at the bifurcation of the internal and external carotid arteries. They are supplied by the carotid artery and have the highest blood flow and O_2 consumption per tissue weight of any organ. As such, they are reporters of blood P_{O_2} rather than O_2 content. Carotid bodies are ideally positioned to monitor O_2 delivery to the brain and are the main, if not only, sensor of acute and chronic arterial hypoxemia in the adult (Milsom and Burleson, 2007). Type I glomus cells are the primary chemosensory cell. Although chemoreceptor sensitivity varies from species to species, there is also considerable difference depending on the site of measurement; chemoreceptor discharge is half-maximal when arterial P_{O_2} is around 55 mmHg which is equivalent to 18 mmHg in the vicinity of the type I cells (Kumar, 2007). Intracellular P_{O_2} is probably considerably lower. Acetylcholine and ATP are the primary secretory products, although the carotid body has been reported to have as many neurotransmitters as the brain (Prabhakar, 2006). Arterial hypoxia initiates an increase in ventilation and peripheral vasoconstriction, whereas if breathing is not possible, a fall in P_{O_2} produces a reflex bradycardia and peripheral vasoconstriction (Marshall, 1994).

Chromaffin cells

Chromaffin cells are associated with systemic veins in ancient fishes and the posterior cardinal veins of teleosts. Catecholamines are secreted in response to hypoxemia and stimulate cardiorespiratory responses as well as increase O_2 transport by red blood cells (Perry and Thomas, 1991), but this may not reflect direct O_2 sensing by chromaffin cells (Perry et al., 2000). Conversely, hypoxemia stimulates mammalian chromaffin cells in the adrenal medulla to release catecholamines into the bloodstream where they have general cardiorespiratory effects (Nurse et al., 2006).

Monitors of regional/local hypoxia

It is well known that in mammals hypoxia contracts pulmonary and relaxes systemic vessels. Although these responses may be modulated by a variety of factors such as paracrine signals from the endothelium or surrounding tissue, the basic responses are intrinsic to the vascular smooth muscle cells (VSMCs) (Madden et al., 1992). Hypoxic pulmonary vasoconstriction (HPV) decreases perfusion of poorly ventilated regions of the lung and thus decreases the potential for partial unsaturation of pulmonary venous (systemic arterial) blood. Hypoxic vasodilation increases tissue perfusion thereby increasing O_2 delivery. Perfused gills and lungs of non-mammalian vertebrates also exhibit hypoxic vasoconstriction, but surprisingly, hypoxia also constricts many systemic conductance (large) vessels in a range of vertebrates from hagfish and lamprey to birds (see Russell et al., 2007). The physiological significance of this is unclear and the effects of hypoxia on resistance-size (0.01–0.2 mm diameter) vessels in many of these animals remains to be determined.

Mechanism of oxygen sensing

Potassium channels

Most effort in identifying the oxygen sensor(s) has focused on mammalian chemoreceptors (carotid body and neuroepithelial bodies) and on pulmonary and systemic arteries. It was thought that since cell activation ultimately appeared to depend on an increase in intracellular calcium, the latter could be achieved through cell depolarization and an increase in calcium influx through voltage-sensitive (L-type) calcium channels. As this can be accomplished by closing potassium channels, it was logical to look for O_2-sensitive potassium channels, and in fact a number of these have been identified in most if not all O_2-sensing tissues, including the carotid and neuroepithelial bodies (Pérez-García et al., 2004;

López-López and Pérez-García, 2007), adrenal chromaffin cells (Nurse et al., 2006), pulmonary artery (Weir and Archer, 1995; Murry et al., 2006) and even fish gill neuroepithelial cells (Jonz et al., 2004; Burleson et al., 2006). Conversely, hypoxic vasodilation (relaxation) has been proposed to result from the metabolism-sensitive potassium channels (K_{ATP} channels) that open during hypoxia (Weir and Archer, 1995), or a loss of O_2-sensitive $k_V1.5$ and $k_V2.1$ channels (Thorne et al., 2002) that hyperpolarize the cell and decrease calcium influx).

Beyond potassium channels

It has become evident, however, that while O_2-sensitive K^+ channels may contribute to, or modulate the hypoxic response, other factors 'upstream' from these or other K^+ channels most likely couple hypoxia to K^+ channel inactivation (Peers and Kemp, 2001; Peers and Kemp, 2004). Although there are exceptions (see below).

In carotid body type I cells hypoxia has been reported to activate NADPH oxidase and increase reactive oxygen species (ROS), activate AMP-activated protein kinase, inhibit mitochondrial metabolism, or stimulate hemeoxygenase-2 (HO-2) production of carbon monoxide, and any of these could serve as the O_2-sensing mechanism (Peers and Kemp, 2001; Dinger et al., 2007; Gonzalez et al., 2007; Lahiri et al., 2006; Prabhakar, 2006; Riesco-Fagundo et al., 2001; Kemp, 2005; Wyatt and Evans, 2007; Wyatt et al., 2007). In airway NEB, hypoxia has been proposed to inactivate NADPH oxidase thereby decreasing ROS (Kemp et al., 2002; Nurse et al., 2006), and in adrenal chromaffin cells a mitochondrial mechanism decreases ROS (Nurse et al., 2006). Pulmonary arteries have perhaps the greatest cadre of potential O_2-sensing mechanisms including redox sensors (Archer and Michelakis, 2002), either an increase or decrease in ROS (Ward et al., 2006), NADPH and NADPH oxidase (Jones et al., 2000; Wolin et al., 2005), cyclic ADP ribose (Evans and Dipp, 2002) and Rho kinase (Fagan et al., 2004). Other upstream activators of hypoxic vasodilation, which may or may not be associated with a decrease in intracellular calcium (Thorne et al., 2004), include intracellular acidosis (Nagesetty and Paul, 1994), redox control of cytosolic NADPH (Wolin et al., 2005), direct modulation of internal Ca^{2+} stores and Rho kinase (Thorne et al., 2004).

Can anyone be right?

The variety of potential O_2-sensing systems suggests that either this process evolved numerous times and now constitutes a highly redundant system, or that perhaps many of these mechanisms may be either modulators of, or even peripheral to, an even more fundamental process. Not surprisingly, many of these proposed O_2-sensing mechanisms have their detractors and in many instances there are as many arguments against a particular sensing mechanism as there are arguments for it. This has fostered reviews such as 'Detecting acute changes in oxygen: will the real sensor please stand up?' (Kemp, 2006), 'Hypoxic pulmonary vasoconstriction: mechanisms and controversies' Aaronson et al., 2006), 'Hypoxic pulmonary vasoconstriction is/is not mediated by increased production of reactive oxygen species' (Ward et al., 2006), 'Oxidant and redox signaling in vascular oxygen-sensing mechanisms: basic concepts, current controversies, and potential importance of cytosolic NADPH' (Wolin et al., 2005), 'The mechanisms of hypoxic pulmonary vasoconstriction: potassium channels, redox oxygen sensors, and controversies' (Archer and Michelakis, 2002), 'Hypoxia, anoxia, and O_2 sensing: the search continues' (Schumacker, 2002), 'Mechanisms of hypoxic pulmonary vasoconstriction: can anyone be right? (Ward and Aaronson, 1999). Are there multiple and redundant O_2 sensors, or does the lack of unequivocal support for a unifying mechanism suggest that it remains to be identified?

Hydrogen sulfide as an oxygen sensor

Peers and Kemp (Peers and Kemp, 2001) state, regarding airway (neuroepithelial) and carotid chemoreceptors, that '...putative O_2 sensors would be drawn from a pool of proteins that naturally underwent oxido-reductive transitions.' However, it is not exactly clear to me why the O_2 sensor must be a macromolecule. In fact we have recently proposed a novel O_2-sensing mechanism that is based on the metabolism of a very simple (but noticeable) gas, hydrogen sulfide (H_2S). This mechanism has both experimental and anecdotal support, as described in the following paragraphs.

Hydrogen sulfide as a biological stimulant

There is a rapidly accumulating body of evidence linking H_2S with nitric oxide (NO) and carbon monoxide (CO) as the third 'gasotransmitter' (Wang, 2002). Reports of the physiopharmacological effects of H_2S suggest an almost ubiquitous presence in tissues. H_2S has been implicated in neurological, cardiovascular, gastrointestinal, genitourinary and endocrine systems where it may have modulatory and/or cytoprotective effects (Qu et al., 2007; Li and Moore, 2008; Leffler et al., 2006; Li and Moore, 2008; Łowicka and Bełtowski, 2007; Szabó, 2007). H_2S has also been proposed to be both pro- and anti-inflammatory (Li et al., 2006; Wallace, 2007) and it has even been reported to induce suspended animation in mice (Blackstone et al., 2005). In the cardiovascular system, H_2S has long been known to dilate systemic vessels (Hosoki et al., 1997). H_2S is synthesized in many, if not all, tissues by two cytosolic pyridoxyl 5′phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine λ-lyase (CSE). CBS is thought to be the primary source for H_2S production in the brain, and CSE in the vasculature (Szabó, 2007; Li and Moore, 2008).

Evidence supporting hydrogen sulfide mediation of vascular hypoxic responses

During the course of independent studies on the phylogeny of vascular responses to hypoxia (Smith et al., 2001; Olson et al., 2001; Russell et al., 2001; Russell et al., 2007) and H_2S (Dombkowski et al., 2004; Dombkowski et al., 2005; Olson, 2005), it became evident that these two stimuli evoked the same response in isolated blood vessels, irrespective of whether the response was a contraction, relaxation or was multi-phasic (Fig. 1). This prompted further investigation into whether this was coincidental or if the two stimuli were somehow related. We (Olson et al., 2006) subsequently showed that, (1) H_2S was enzymatically generated by many blood vessels, (2) inhibition of H_2S production inhibited vascular responses to hypoxia (Fig. 2), (3) addition of cysteine, the metabolic precursor for H_2S, greatly augmented the hypoxic response, (4) hypoxia and H_2S appeared to compete for a common downstream activation process, and (5) hypoxia and H_2S produce the same degree of cellular depolarization in vascular smooth muscle. We also found evidence for H_2S mediation of hypoxic relaxation of the trout urinary bladder (Dombkowski et al., 2006), suggesting that this mechanism may be intrinsic to smooth muscle in general.

These studies have led to the formulation of a model for O_2 sensing through O_2-dependent regulation of H_2S metabolism (Olson et al., 2006). In this model (Fig. 3) H_2S is constitutively

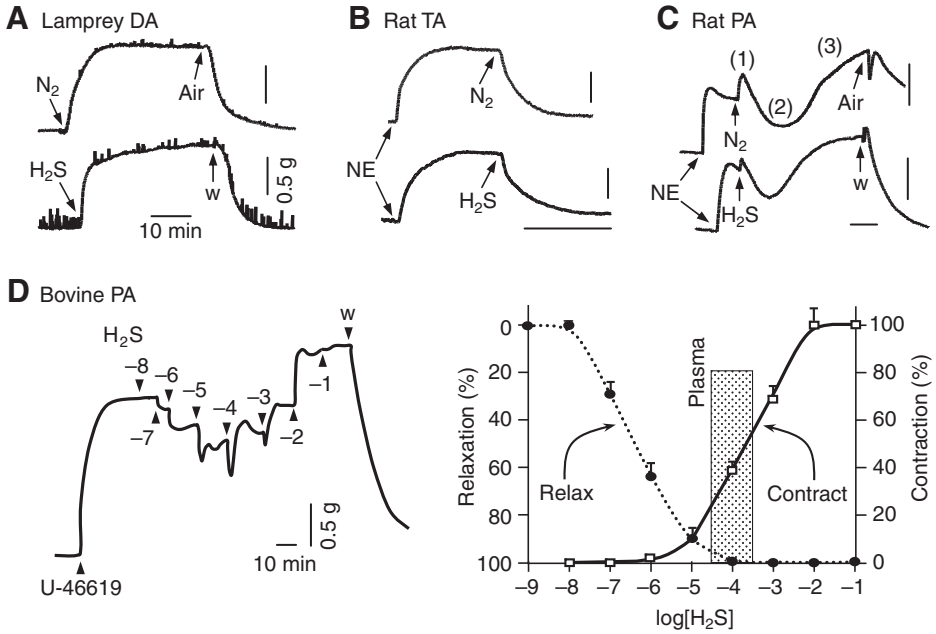

Fig. 1. Hypoxia (100% N_2) and H_2S (1 mmol l^{-1}; as total sulfide, H_2S and HS^-) produce identical contractions in lamprey dorsal aorta (DA; A), relaxations in norepinephrine (NE, 10^{-6} mol l^{-1}) pre-contracted rat thoracic aorta (TA; B), and triphasic contraction–relaxation–contraction (1, 2, 3) in norepinephrine pre-contracted rat pulmonary artery (PA; C). Air indicates return to normoxia; w, wash. In U-46619 (thromboxane A_2 mimetic, 10^{-7} mol l^{-1}) pre-contracted bovine pulmonary artery (PA; D) H_2S produces relaxation at low concentrations and contractions at higher concentrations. The left panel shows an individual trace, and in the right panel the two phases are separated into individual dose–response curves (N=8); stippled rectangle indicates range of reported [H_2S] (measured in mol l^{-1}) in rat plasma. Horizontal time bar in all figures is 10 min, vertical tension scale is 0.5 g. Adapted from Olson et al. (Olson et al., 2006) with permission.

synthesized in the cell cytoplasm and oxidized in the mitochondria. The actual concentration of biologically active H_2S is determined by the simple balance between H_2S production and the amount of O_2 available for H_2S oxidation, i.e. tissue P_{O_2}. In addition CBS and CSE also appear to be sensitive to P_{O_2} or intracellular redox state (Maclean and Kraus, 2004; Stipanuk, 2004; Banerjee and Zou, 2005) and may either directly contribute to intracellular [H_2S] or may provide a long-term mechanism to bias the rate of constitutive H_2S production. We recently found evidence coupling H_2S production and metabolism to O_2. As shown in Fig. 4A,B, H_2S production by minced trout heart (at P_{O_2}~0) is increased by cysteine (Cys) and transiently inhibited by injection of micromolar amounts of O_2. Because this only occurs at very low P_{O_2}, it suggests that under normal circumstances intracellular H_2S is very low. Conversely, there is a net H_2S consumption by purified trout mitochondria that is increased in the presence of O_2 and decreased when the mitochondria are gassed with nitrogen (N_2) or heat denatured (Fig. 4C,D). Hildebrandt and Grieshaber (Hildebrandt and Grieshaber, 2008) have recently summarized the pathways involved in mitochondrial H_2S oxidation.

Most of our attention to date has focused on H_2S metabolism as the mediator of hypoxic vasoconstriction. However, in many vessels, i.e. trout efferent branchial arteries (Dombkowski et al., 2004) toad and duck pulmonary arteries (Dombkowski et al., 2005) and bovine pulonary arteries (Fig. 1D), it appears that H_2S produces both vasoconstriction and vasodilation and that these occur at different H_2S concentrations. This implies that H_2S has multiple effects on the mechanical properties of vascular smooth muscle, a rare but not unheard of attribute. This too may have physiological significance. For example, it is predicted that low concentrations of H_2S, which would be expected in normoxia, may in fact aid in pulmonary vasodilation; this would then convert to pulmonary vasoconstriction when O_2 levels fall and [H_2S] increases.

Arguments that H_2S-mediated contraction of vascular smooth is only an indirect response have been presented by Koenitzer et al. (Koenitzer et al., 2007). They observed that a H_2S-mediated dilation of rat aortas became more sensitive to H_2S at low P_{O_2}, whereas a H_2S-mediated contraction was only observed at high [H_2S] with concomitantly high P_{O_2}. They proposed that the H_2S-mediated contraction was due to an oxidation product of H_2S rather

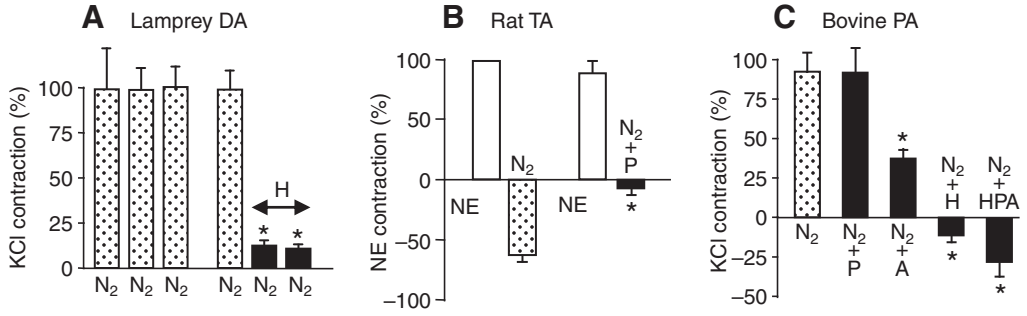

Fig. 2. Inhibition of hypoxic responses by inhibitors of H_2S synthesis. (A) Three consecutive exposures to hypoxia (N_2) produce identical contractions in lamprey dorsal aorta (DA; left bars) whereas in parallel experiments the second and third hypoxic contractions are significantly (*) inhibited after addition of hydroxylamine (H), an inhibitor of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). (B) Hypoxic relaxation of a norepinephrine-contracted (NE; 10^{-6} mol l^{-1}) rat thoracic aorta (TA) is significantly (*) inhibited by the CSE inhibitor propargyl glycine (P). (C) Hypoxic contractions of bovine pulmonary arteries (PA) are not affected by propargyl glycine (P) but are increasingly inhibited by the CBS inhibitor aminooxy acetate (A), hydroxylamine (H), or a combination of all three inhibitors (HPA). Means ± s.e.m.; responses are normalized to an initial KCl (80 mmol l^{-1}) or NE contraction. (Adapted from Olson et al., 2006.)

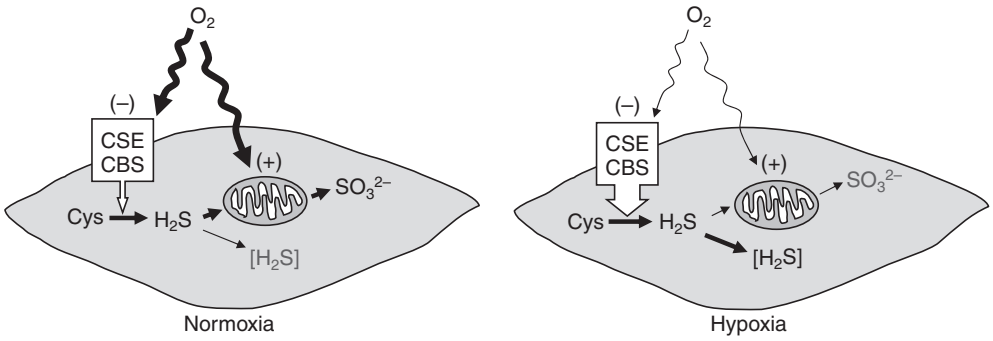

Fig. 3. Proposed mechanism of O_2 sensing *via* H_2S metabolism. H_2S is constitutively produced from cysteine (Cys) metabolism in the cytosol. During normoxia (left panel) H_2S is continuously oxidized to sulfite in the mitochondria thereby maintaining low intracellular [H_2S]. A fall in oxygen availability (right panel) decreases mitochondrial H_2S oxidation resulting in an increase in biologically active [H_2S] and initiation of hypoxic responses. The enzymes generating H_2S from cysteine, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) also have the potential of O_2 sensitivity, thereby enabling either short-term regulation of [H_2S] or placing a long-term bias on the rate of H_2S metabolism.

than a direct vasoconstrictory action of H_2S itself, implying that H_2S is only a vasodilator. However, rat aortas also dilate to hypoxia and may not be a good model with which to examine H_2S vasoconstriction. We (Olson et al., 2008a) repeated these studies in both hagfish and lamprey dorsal aortas, where hypoxia and H_2S only produce vasoconstriction and found that these vessels also became more sensitive to H_2S at low P_{O_2}. Thus it is evident that the H_2S contraction is not the result of an oxidation product. This also provides further support for our hypothesis of H_2S in O_2 sensing, i.e. the vessels become more sensitive to exogenous H_2S at low P_{O_2} because of the additive affect of endogenous H_2S production.

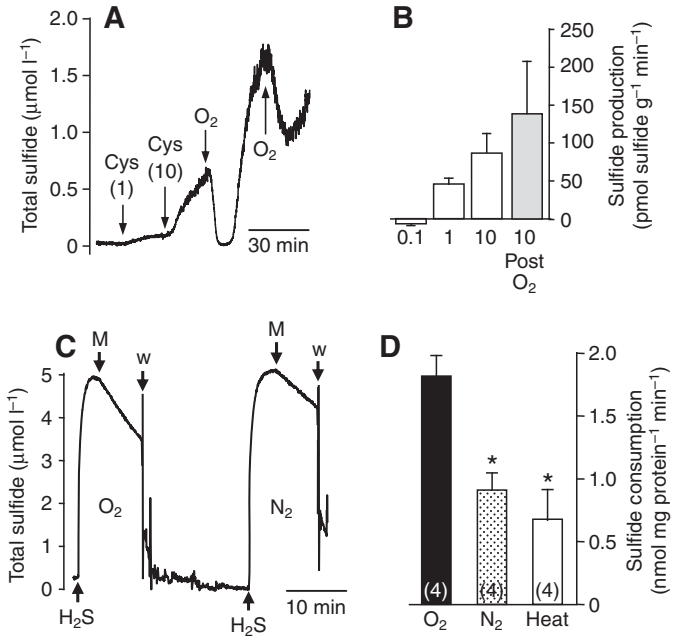

Fig. 4. O_2-dependent H_2S production and consumption measured in real-time with a polarographic H_2S sensor. (A,B) H_2S production (as total sulfide) by minced trout heart increases with increasing concentrations of cysteine (mmol l^{-1}) at P_{O_2}~0 and is transiently inhibited by injection of oxygen (O_2) into the reaction chamber. H_2S production resumes, presumably after the oxygen has been depleted (post O_2). (A) A single representative trace, (B) pooled results expressed as means ± s.e.m. (*N*=4). (C,D) H_2S is consumed by addition of purified trout heart mitochondria (M). The rate of sulfide consumption is greater when the reaction mixture is equilibrated with room air (O_2) than with nitrogen (N_2) or if the mitochondria are heat denatured (Heat). w, wash. (C) Single representative trace, (D) pooled results expressed as means ± s.e.m. (*N*=4 replicates). A, B adapted from Whitfield et al. (Whitfield et al., 2008), with permission; C, D adapted from Olson et al. (Olson et al., 2008b), with permission.

Hydrogen sulfide in chemoreception

If H_2S metabolism is involved in O_2 sensing in vascular smooth muscle, we wondered if this could be a general O_2-sensing mechanism in other oxygen-sensitive tissues as well. To examine this, we turned to the external chemoreceptors on the first gill arch of trout. We (Olson et al., 2008b) found that injection of H_2S into the buccal cavity mimicked the classical hypoxic induced bradycardia (Fig. 5) and this response was inhibited when the first, but not second, gill arches, were removed. In addition, both hypoxia and H_2S depolarized isolated zebrafish neuroepithelial cells. This suggests that H_2S metabolism may be an O_2-sensing mechanism in a variety of O_2-sensitive tissues. Interestingly, we also found in unanesthetized trout that whereas hypoxia increased plasma sulfide when measured with a sulfide electrode, it did not affect plasma H_2S measured with a polarographic H_2S sensor. This suggests that some sulfide moiety is carried in the plasma, but it is not H_2S. This is considered further under Curbing the exuberance (below).

Anecdotal support for hydrogen sulfide in oxygen sensing

There is some anecdotal support for the involvement of H_2S in vascular signaling and in hypoxia sensing in general. First, H_2S has been a common environmental gas since the origin of life and cells have had considerable opportunity to use this gas as a metabolic substrate and deal with potential toxicity (Goubern et al., 2007). Second, there is evidence to support intracellular compartmentalization of H_2S production and oxidation. Searcy (Searcy, 2003) proposed that mitochondria originated from sulfide (H_2S) oxidizing thermophilic bacteria and the nucleocytoplasm from sulfide-reducing Archaea. Thus the mechanism for constitutive production of H_2S and its O_2-dependent inactivation by mitochondria is a common feature of all eukaryotic cells. Third, systemic arterioles consume O_2 at a rate of up to 500 times that of resting skeletal muscle (Shibata et al., 2005). This is far greater than that required to maintain wall tension and the reason for this high level of O_2 consumption is not readily apparent. Could it be due to the continual need for H_2S oxidation in these cells?

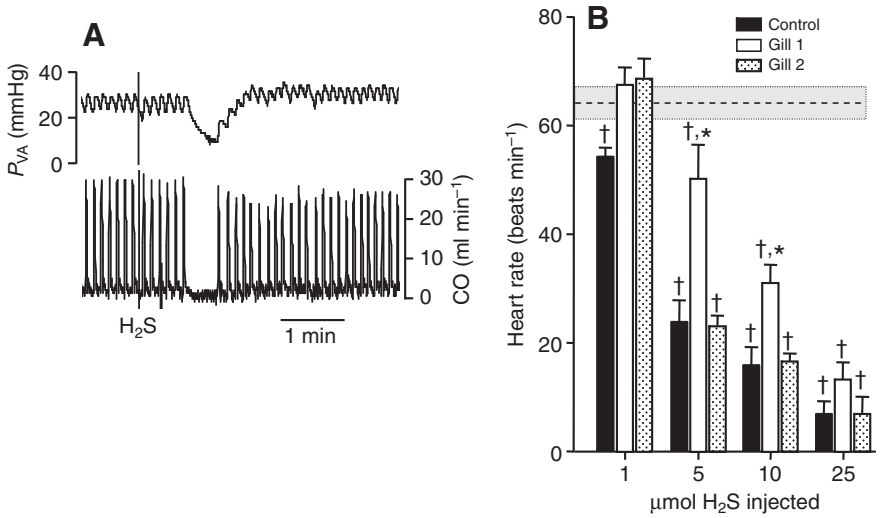

Fig. 5. Evidence for H_2S involvement in trout gill chemoreceptors. (A) Injection of 5 μmol H_2S into the buccal cavity of an unanesthetized 600 g trout previously implanted with a pressure cannula in, and flow probe around, the bulbus arteriosus produces a bradycardia within 5 s that mimics a hypoxic bradycardia. P_{va}, ventral aortic pressure; CO cardiac output. (B) Heart rate following intrabuccal injection of a 1 ml bolus of H_2S in intact trout (black bars; N=13–15) or trout with either the first (Gill 1; white bars; N=7) or second (Gill 2; stippled bars; N=6) pair of gills removed. Dashed lines indicate mean ± s.e.m. heart rate of all fish prior to H_2S. H_2S produced a dose-dependent bradycardia in control trout that was similar to that produced in trout with the second pair of gills removed. Removal of the first pair of gills decreased H_2S sensitivity. †Significantly different from pre-H_2S; *significantly different from intact trout after H_2S injection. The bradycardia at 5 and 10 mmol l^{-1} is significantly (*) attenuated in trout with the first pair of gills removed relative to the two other groups. Adapted from Olson (Olson et al., 2008b), with permission.

Curbing the exuberance

Like its predecessor nitric oxide (NO), H_2S has garnered considerable interest as a biologically important regulatory molecule in a seemingly endless number and variety of physiological processes and for its potential therapeutic value (Szabó, 2007). Before we get swept away in all the excitement we need to examine several aspects of H_2S signaling that are a bit troublesome.

The first concerns reported levels of H_2S in plasma. In the approximately 25 studies conducted since 2000 all but one have reported plasma or blood H_2S in micromolar concentrations (typically 30–60 μmol l^{-1} but as high as 300 μmol l^{-1}) and all but one have used acidic or alkaline modification of the sample prior to measurements [summarized in Whitfield (Whitfield et al., 2008) and detailed in the supplementary material]. These concentrations are typically used in physiological studies, however, they are tens to hundreds of times greater than that which inhibits purified cytochrome *c* oxidase (1 μmol l^{-1}). Furthermore, at these concentrations, the smell of H_2S should be readily apparent in both plasma and exhaled breath, which, fortunately, is not the case (Whitfield et al., 2008). These 25 studies have also largely ignored reports going back as far as the 1920s (c.f. Haggard, 1921) that showed very little, or no, H_2S in blood and that demonstrated a large capacity for blood to absorb exogenously administered H_2S [summarized by Whitfield (Whitfield et al., 2008)]. We (Whitfield et al., 2008) used a newly developed polarographic sensor to measure H_2S gas in plasma or whole blood at sub-micromolar concentrations, and without any chemical modification, and failed to find measurable amounts of H_2S in blood or plasma from lamprey, trout, mouse, rat, pig or cow, or in an unanesthetized trout

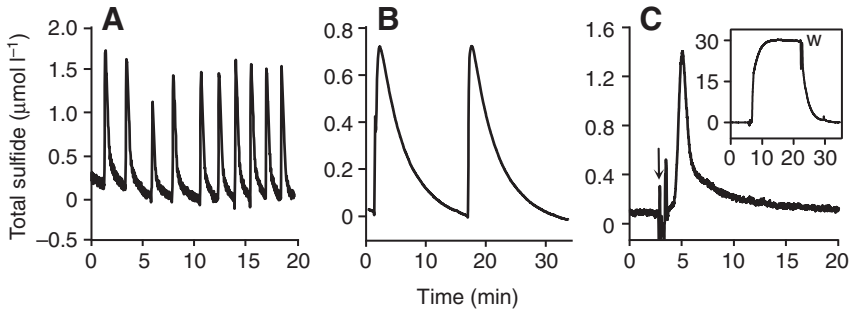

Fig. 6. Exogenous H_2S is rapidly consumed by trout (A) or rat (B) blood *in vitro* effectively maintaining H_2S concentration close to 0. The concentration of H_2S (as total sulfide) was measured in real-time with a polarographic H_2S sensor. Serial additions of H_2S (as Na_2S), each sufficient to raise total sulfide to 10 μmol l^{-1}, did not increase sulfide by more than ~1.5 μmol l^{-1}. The rate of sulfide consumption in trout blood was tenfold greater, presumably due to the presence of mitochondria in trout erythrocytes. (C) Effect of exogenous sulfide (as Na_2S) on sulfide concentration of dorsal aortic blood in an unanesthetized trout. An extracorporeal pump circulated blood from the dorsal aorta across the sensor and returned it to the caudal vein. The arrow indicates bolus injection of Na_2S into the caudal vein cannula. The amount of Na_2S injected was theoretically sufficient to raise plasma sulfide to 30 μmol l^{-1} when fully mixed in the plasma. Inset shows injection of the same amount of sulfide into a recirculated volume of Hepes buffer equivalent to the trout's plasma volume and pH. Sulfide is rapidly removed from the plasma *in vivo*, but not from the buffer. w, wash. Adapted from Whitfield et al. (Whitfield et al., 2008), with permission.

fitted with an extracorporeal loop. Furthermore, exogenously administered H_2S rapidly disappeared from whole blood in vitro (Fig. 5A,B) and from blood of the unanesthetized trout (Fig. 5C). We also found that the antioxidant buffer commonly employed with S^{2-}-selective electrodes generated H_2S from bovine serum albumin, presumably from hydroxyl replacement of cysteine sulfur. Our studies with the polarographic H_2S sensor raise the question of the physiological relevance of studies that employ what appears to be pharmacological concentrations of H_2S. What is too high, and what is physiological in the region of the intracellular effectors? Although it is clear that H_2S does not circulate at $30\,\mu mol\,l^{-1}$, it remains to be determined how fast exogenous H_2S moves across the cell membrane and how it is distributed within the cell. Does it equilibrate, or does metabolism maintain a lower intracellular concentration, and is H_2S partitioned within the cell? Are seemingly excessive exogenous H_2S concentrations pharmacological or are they necessary to produce a physiological increase in intracellular H_2S? Toxicological studies lend some credence to the latter. Purified cytochrome *c* oxidase is inhibited by nanomolar H_2S concentrations whereas mitochondria and whole animals are poisoned at micromolar concentrations and sulfide oxidizing bacteria survive $1–2\,mmol\,l^{-1}$ H_2S (Bagarino, 1992). (In our experience some vessels even tolerate $>10\,mmol\,l^{-1}$ H_2S.) Because there does not appear to be any species-specific differences in cytochrome *c* oxidase (Bagarino, 1992), it seems likely that differential rates of H_2S metabolism could account for the wide range in H_2S sensitivity, and perhaps, the threshold concentration necessary for physiological responses. Clearly, one of the next breakthrough in H_2S physiology will be measurement of H_2S concentration at the sub-cellular level. Our studies showing rapid removal of exogenous sulfide from the blood, *in vivo* and *in vitro* (Whitfield et al., 2008) also raises the question of how numerous studies could show that a variety of experimental manipulations have had lasting effects on plasma [H_2S].

The second troublesome aspect of H_2S signaling concerns the lack of specific inhibitors of CBS and CSE. The only readily available enzyme inhibitors, aminooxyacetate (AOA) for CBS, propargylglycine (PPG) and β-cyanoanalyine (BCA) for CSE and hydroxylamine (HA) for both CBS and CSE lack specificity and are often poorly absorbed by intact tissues (Szabó, 2007). Thus ascribing definitive physiological effects to inhibition of H_2S production is somewhat tenuous.

The third aspect is that H_2S is a good reductant and can potentially react with other signaling molecules (i.e. NO) potentially removing them or modifying their biological attributes. Thus it is not always evident if the effect of H_2S is direct or indirect, although this may not be critical.

Conclusions and future directions

Hypoxia, be it of external or internal origin is a potential threat to all animals. Oxygen-sensing abilities of a variety of specialized cells in vertebrate tissues have been known for nearly one hundred years, but the identity of the intracellular Oxygen-sensing mechanism has not yet been resolved. The lack of a unifying hypotheses of O_2-sensing signal transduction has left the door open for yet another potential O_2 sensor; this one based on the balance between constitutive production of biologically active H_2S and its metabolism by available O_2. The role of H_2S as a 'gasotransmitter' in a seeming endless list of biological mechanisms including O_2 sensing needs additional validation. Important questions regarding the biology of H_2S remain. (1) Does H_2S circulate in the blood bound to some carrier or is it completely metabolized? It is difficult to reconcile studies with the polarographic sensor with those that use other methods and report a change in plasma [H_2S] following experimental manipulation. (2) What is the mechanism of H_2S action in excitable cells? To date, the only known effect is *via* opening of K_{ATP} channels, and that relaxes vascular smooth muscle. (3) What is the biologically active form of H_2S? As a weak acid with a p*K*a close to 7 (Olson, 2005), H_2S in solution exists as both H_2S gas and HS^-. As an anion, HS^- is very similar in size to Cl^- and could potentially become involved in Cl^--mediated processes. (4) Does H_2S participate in O_2 sensing in other oxygen-sensitive tissues, i.e. is there a universal O_2 sensor, or did O_2-sensing mechanisms evolve independently with different tissues? Can H_2S exemplify Occam's razor?

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