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Review

Oxygen sensors in context

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Abstract

The ability to adapt to changes in the availability of O_2 provides a critical advantage to all O_2-dependent lifeforms. In mammals it allows optimal matching of the O_2 requirements of the cells to ventilation and O_2 delivery, underpins vital changes to the circulation during the transition from fetal to independent, air-breathing life, and provides a means by which dysfunction can be limited or prevented in disease. Certain tissues such as the carotid body, pulmonary circulation, neuroepithelial bodies and fetal adrenomedullary chromaffin cells are specialised for O_2 sensing, though most others show for example alterations in transcription of specific genes during hypoxia. A number of mechanisms are known to respond to variations in PO_2 over the physiological range, and have been proposed to fulfil the function as O_2 sensors; these include modulation of mitochondrial oxidative phosphorylation and a number of O_2-dependent synthetic and degradation pathways. There is however much debate as to their relative importance within and between specific tissues, whether their O_2 sensitivity is actually appropriate to account for their proposed actions, and in particular their modus operandi. This review discusses our current understanding of how these mechanisms may operate, and attempts to put them into the context of the actual PO_2 to which they are likely to be exposed. An important point raised is that the overall O_2 sensitivity (P50) of any O_2-dependent mechanism does not necessarily correlate with that of its O_2 sensor, as the coupling function between the two may be complex and non-linear. In addition, although the bulk of the evidence suggests that mitochondria act as the key O_2 sensor in carotid body, pulmonary artery and chromaffin cells, the signalling mechanisms by which alterations in their function are translated into a response appear to differ fundamentally, making a global unified theory of O_2 sensing unlikely.

Keywords: Oxygen sensor; Mitochondria; Carotid body; Pulmonary circulation; Oxygen distribution; Hypoxic pulmonary vasoconstriction; Electron transport chain; Reactive oxygen species; Redox state

1. Introduction

Oxygen is the stuff of life. In aerobic respiration it is used as the ultimate electron acceptor at the end of a series of oxidation–reduction reactions that drive production of the ubiquitous high energy compound ATP, the immediate source of energy for cell function. O_2 is also utilised as a substrate for the synthesis or degradation of numerous cellular constituents, including many signalling mediators. Evolution has adapted air-breathing animals to perform optimally with an ambient PO_2 of ~21 kPa at sea level, though cellular O_2 consumption coupled with the constraints of diffusion through the tissues means that the intracellular PO_2 may be ~10% of this, and less in highly metabolically active tissues. Moreover, whilst the arterial PO_2 in mammalian adults is ~13 kPa, in the fetus it is ~5 kPa. Hypoxia is thus a relative term, and is most usefully defined as a condition in which failure of either delivery or use of O_2 limits normal tissue function. Respiratory and cardiovascular diseases are commonly associated with hypoxaemia and tissue hypoxia, which also occur at altitude. A number of mechanisms allow the body to maintain adequate O_2 delivery to the tissues and to adapt to reduced O_2 availability.

Several classical tissues are recognised for their specialised response to acute hypoxia [1]. Carotid body glomus (type I) cells stimulate ventilation in response to decreased arterial PO_2. Pulmonary arteries constrict to localised hypoxia (hypoxic pulmonary vasoconstriction, HPV), thereby optimising ventilation–perfusion matching and gas exchange, whereas systemic arteries dilate, increasing O_2 delivery. Neuroepithelial bodies (NEBs), clusters of cells exposed to the airway lumen at branch points, are innervated by the vagus and release neurotransmitters in

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response to hypoxia; they may therefore sense airway hypoxia, and it has been suggested that they may act in concert with the carotid body and HPV, especially during the transition to air-breathing at parturition [2]. Fetal and neonatal adrenomedullary chromaffin cells (AMCs) release catecholamines in response to hypoxia, probably an important protective mechanism during parturition that supports cardiac function and transformation of the airway epithelium for air-breathing [3]. Notably, the final response to hypoxia in glomus cells, NEBs and AMCs involves inhibition of K^+ channels, depolarisation, voltage-gated Ca^{2+} entry and release of neurotransmitters [4]. The situation in pulmonary artery smooth muscle cells (PASMCs) is more complex and controversial, with HPV probably involving both voltage-dependent and -independent Ca^{2+} entry, Ca^{2+} release from ryanodine-sensitive stores, and Rho kinase-mediated Ca^{2+} sensitisation [5]. Unlike HPV, hypoxic systemic vasodilatation is often driven by the O_2 requirements of the perfused tissue, and metabolites or mediators produced by the surrounding cells. Nevertheless, isolated systemic arteries do dilate to hypoxia, though myriad mechanisms have been implicated in this response, including effects on ion channels, intracellular Ca^{2+}, pH and phosphate, Ca^{2+} sensitivity and cross-bridge cycling [6–9]. It is therefore unwise to consider hypoxic systemic vasodilatation as a single phenomenon.

It is likely that most other cell types also respond to hypoxia, especially if it is prolonged. Hypoxia causes increased transcription of a large number of hypoxia-sensitive genes, leading for example to increased production of erythropoietin in the renal cortex and consequently an increase in red cell mass, and increased expression of growth factors such as vascular endothelial growth factor (VEGF), a key driver of angiogenesis. Hypoxia also modulates the balance between aerobic and anaerobic (glycolytic) energy production in numerous tissues, so reducing O_2 demand at the expense of reduced efficiency of substrate use.

The existence of these homeostatic processes obviously implies an ability to sense deviations in $[O_2]$ from the optimum, and/or the metabolic consequences of such deviations. It is now recognised that a number of mechanisms with different sensitivities may effectively act as O_2 sensors (though it may not always be O_2 that is actually sensed) and coexist in the same cell, though there is controversy as to their relative importance and signalling pathways between and within individual tissues, and potentially between species [1,4,10,11]. An important consideration when determining the physiological role of such sensors is how well their O_2 sensitivity matches that of their effector response(s). In this review I discuss the mechanisms of several proposed O_2 sensors, and attempt to correlate their apparent O_2 sensitivity to that of their effectors, and indeed to the likely range of PO_2 present in the tissue or location of interest. In broad terms O_2 sensor mechanisms can be differentiated into those dependent on perturbations of mitochondrial function and energy state (bioenergetic), and those on perturbations of O_2-dependent synthesis or degradation of mediators (biosynthetic), though in places this distinction becomes blurred.

Note that throughout this review I use the term P50 rather than K_M to denote the PO_2 causing half-maximal responses of O_2 sensitive mechanisms, as the response may not always strictly adhere to Michaelis–Menten kinetics. I have followed the convention of using PO_2 instead of the technically more correct $[O_2]$, as the former is more immediately comprehensible. However, as biochemical determinations of K_M are usually given in terms of concentration, the P50s of the putative O_2 sensors are shown in terms of both PO_2 and $[O_2]$.

2. Bioenergetic O_2 sensing mechanisms

2.1. Mitochondria

Mitochondria are the largest consumers of O_2, and as such are the key determinants of cytosolic PO_2 and the O_2 gradient between alveoli and cytosol. They are also recognised as important signalling organelles [12]. Mitochondria are therefore prime contenders for the location of a key O_2 sensor, and over the years a substantial body of evidence has developed supporting this conjecture. There is for example a wide consensus that inhibitors of oxidative phosphorylation or procedures that modify mitochondrial function strongly affect O_2 sensing in PASMCs, glomus cells and AMCs [1,4,13–17], though not in NEBs [2,18]. Significant controversy exists however concerning the signalling mechanisms that link mitochondrial function to the effectors, and there are currently three main hypotheses of mitochondrial O_2 sensing, involving cytosolic redox state, reactive O_2 species (ROS) and energy state respectively (Fig. 1). It is helpful to review mitochondrial function at this point so that these hypotheses can be evaluated in context.

The Kreb's cycle and β-oxidation of fatty acids generate reduced nicotinamide adenosine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) which are oxidised by the electron transport chain (ETC) in the mitochondrial inner membrane. The operation of the ETC is outlined in Fig. 2. Briefly, oxidation of NADH in complex I and $FADH_2$ in complex II leads to transfer of 2 electrons to ubiquinone to form ubiquinol. This is reoxidised by complex III (cytochrome bc_1) in two stages. One electron is first removed by the Rieske Fe–S group leaving ubisemiquinone, and transferred via cytochromes *c1* and *c* to complex IV (cytochrome *c* oxidase, COX). The ubisemiquinone left behind is reoxidised to ubiquinone by cytochrome b_L, which passes the remaining electron to cytochrome b_H. This reduces ubiquinone first to ubisemiquinone and then back to ubiquinol, which re-enters complex III. Oxidation of one molecule of ubiquinol back to ubiquinone thus takes 2 cycles of complex III, with sequential transfer of its 2 electrons to cytochrome *c*. These electrons are finally and sequentially transferred by cytochromes *a* and *a3* in complex IV to O_2 to form water. Operation of complexes I, III and IV cause extrusion of protons thus generating the mitochondrial membrane potential ($\Delta\Psi m$) and proton gradient (ΔpH) which drive the F0F1 ATP synthetase (Fig. 2; [19]). The mechanisms of the ETC are such that at various points single electrons can be lost to molecular O_2 to form reactive O_2 species (ROS) in the form of superoxide, primarily from reduced flavins in complex I and ubisemiquinone in both Qo (intermembrane space) and Qi (matrix) sides of complex III; as much as 3% of electron flux through the ETC may be constitutively lost in this way [20]. Superoxide is

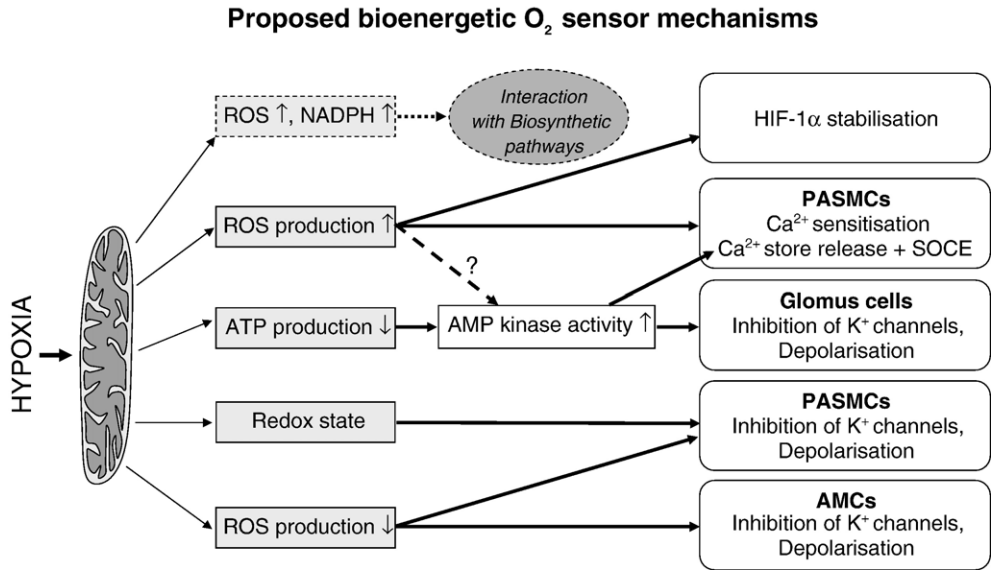

Fig. 1. Summary of bioenergetic O_2 sensor mechanisms mediated by hypoxia-dependent alterations of electron flux through the electron transport change.

rapidly dismuted to the more stable and mobile peroxide by cytosolic and mitochondrial superoxide dismutase (CuZnSOD and MnSOD respectively); if ROS are involved in signalling then peroxide is the likely signalling moiety.

The P50 of cytochrome *aa3* is reported to be ~0.07 kPa PO_2 (~0.7 μM) as measured in cells from systemic tissues [21]. If $[O_2]$ becomes rate limiting all components of the ETC proximal to complex IV will become more reduced, leading to accumulation of upstream reducing equivalents and a more reduced cytosolic redox state, as reflected by an increased ratio of the important redox pair of reduced and oxidised glutathione (GSH/GSSG). ATP production would only be expected to fall if $\Delta\Psi m$ and ΔpH were sufficiently decreased [22]. It should be noted however that the "near equilibrium" hypothesis of Erecinska and Wilson predicts that cytochrome *c* and proximal components of the ETC will become reduced even well above the P50 as the PO_2 decreases, if sufficient reducing equivalents are available as substrate, yet without a decrease in electron transport through COX [23,24]. Consistent with the concept of inhibition of mitochondrial function, increased cytosolic GSH/GSSG and NADH/NAD^+ ratios have indeed been reported in O_2 sensitive tissues during moderate hypoxia [25–27], although others have suggested little change in global redox state [28]. What happens to mitochondrial ROS production in hypoxia is open to question and the source of much debate [28–32]. Whereas a fall in $[O_2]$ might be expected to cause a decrease simply because there is less O_2 from which superoxide can be formed, reduction of the proximal ETC will increase the concentration of electron donors and thus promote electron transfer to O_2; as superoxide production is proportional to $[O_2] \times$ [electron donor] [20], it could therefore theoretically go up or down during hypoxia depending in part on the relative changes to these

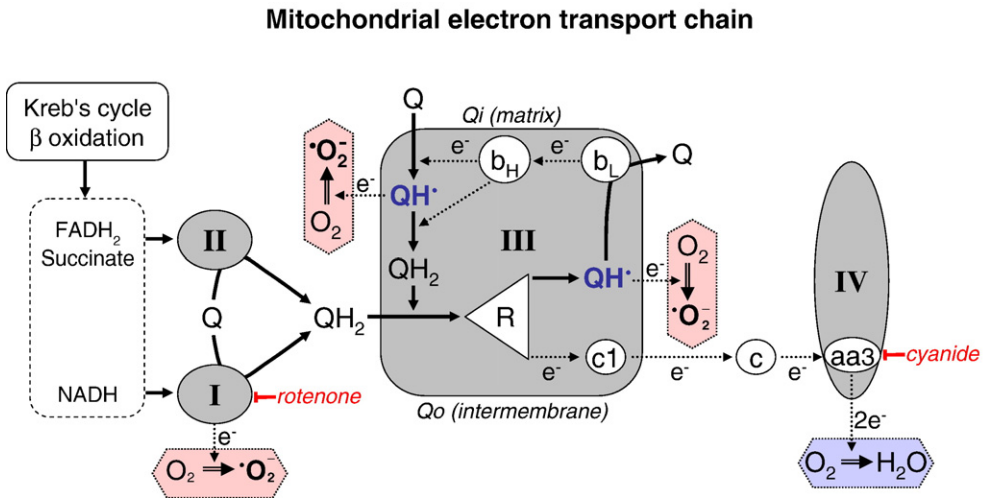

Fig. 2. Basic function of the mitochondrial electron transport chain, illustrating complexes I, II, III and IV, the ubiquinone (Q) cycle and sites of superoxide ($\cdot O_2^-$) production. QH_2, ubiquinol; $QH^{\cdot}$, ubisemiquinone; R, Rieske Fe–S protein; c, cytochrome *c*; c1, cytochrome *c1*; aa3, cytochromes *a* and *a3*; $b_{H/L}$, cytochromes b_H and b_L.

variables. The latter may differ between cell types and may depend on the relative expression of COX to proximal components of the ETC [21]. What happens to mitochondrial ROS production in hypoxia is a critical point, as opposite changes are postulated by two of the three main hypotheses of mitochondrial O_2 sensing.

2.2. Energy state

In investigating the role of mitochondria in O_2 sensing, considerable use has been made of pharmacological inhibitors of the ETC. As discussed above, a large number of studies in several tissues have shown that inhibition of oxidative phosphorylation has profound effects on O_2 sensing, leading to the concept that changes in energy state may underlie the final response to hypoxia. However, there are disparities between tissues as to the precise effects of inhibitors acting at different points in the ETC. In the glomus cell inhibitors of both the proximal (e.g. rotenone, complex I) and distal (e.g. cyanide, cytochrome oxidase) ETC are known to mimic hypoxia and cause inhibition of K^+ channels, as does the F0/F1 synthetase inhibitor oligomycin [14,33,34], leading to the proposal that a fall in ATP production mediates the response. In AMCs however, although rotenone also mimics hypoxia and promotes depolarisation and secretion, cyanide either has no effect or promotes hyperpolarisation and reduces secretion [17], predicating against a signalling role for energy state in this tissue. The situation is less clear in PASMCs, as although there is agreement that proximal ETC inhibitors abolish O_2 sensing and responses associated with HPV, only some reports suggest that they mimic hypoxia [35–37], whereas others are adamant that they do not [15,27,38,39]. Reports on the effects of cyanide on PASMCs are variable, with low concentrations either having no effect or augmenting HPV, and high concentrations abolishing HPV without mimicking hypoxia [15,27,36,38,39]. In addition, we find that oligomycin, like high concentrations of cyanide, abolishes HPV in small pulmonary arteries without causing constriction [40], essentially the opposite of what occurs in glomus cells [14,33]. Again, this would tend to suggest a signalling mechanism that is unrelated to ATP production in PASMCs.

Cytosolic [ATP] is generally well maintained in the face of ADP accumulation by the adenylate kinase reaction, where two ADP are used to produce one molecule of ATP and one of AMP. As the AMP/ATP ratio thus varies close to the square of the ADP/ATP ratio, it is therefore a very sensitive indicator of energy state. AMP kinase is an ubiquitous energy sensor that is activated by the AMP/ATP ratio via a multi-site, synergistic mechanism, making it exquisitely sensitive to energy state [41]. It is known to modulate many cellular functions but in particular ATP production and consumption, including upregulation of glucose uptake and glycolysis in hypoxia [41]. Convincing evidence has recently been presented that in glomus cells AMP kinase provides a direct link between suppression of mitochondrial function by hypoxia and the inhibition of K^+ channels [42,43]. It has also been proposed that AMP kinase sub-serves the same function in PASMCs, but in this case via modulation of Ca^{2+} release from ryanodine-sensitive stores [42]. However, on balance the evidence from mitochondrial inhibitor studies does not appear to be consistent with an energy state based hypothesis either for HPV or AMCs (see above). Glycolysis provides a significant proportion of ATP in PASMCs, especially in hypoxia, and is rate limited by glucose entry [7]. Thus a further point predicating against an energy state/AMP kinase hypothesis for HPV is that the latter is selectively inhibited by hypoglycaemia or inhibition of glycolysis, and potentiated by hyperglycaemia [27,44], the opposite of what would be expected if a fall in ATP production was the initiating signal. In contrast and consistent with the AMP kinase hypothesis, hypoglycaemia potentiates the hypoxic ventilatory response in humans [45]. Indeed, some studies have suggested that the carotid body responds to hypoglycaemia alone and may play a role in glucose homeostasis, though this is not universally accepted [46–49].

2.3. Redox state and ROS

Hypotheses based on redox state and mitochondrial ROS production are associated with effects of mitochondrial inhibition that are unrelated to energy state per se. The Redox hypothesis of HPV developed by Weir and Archer [25,50,51] proposes that during hypoxia inhibition of oxidative phosphorylation and decreased ROS production cause the cytosol of PASMCs to become more reduced, leading to inhibition of redox-sensitive KV channels, depolarisation and Ca^{2+} entry via voltage-dependent Ca^{2+} channels. Inhibition of the proximal ETC would therefore be expected to mimic hypoxia (as indeed reported by supporters of this hypothesis, [35–37]), whereas inhibition of the distal ETC might be expected to suppress the effects of hypoxia, as this promotes ROS production. Studies using exogenous oxidising and reducing agents tend to support this hypothesis, as their reported effects (e.g. modulation of K^+ channel activity) are as predicted if hypoxic-signalling is via a more reduced cytosolic environment [50,52–54]. A similar hypothesis has been proposed for O_2 sensing in AMCs, although in this case there is good evidence that it is the fall in mitochondrial ROS production that causes inhibition of K^+ channels and depolarisation during hypoxia, rather than any change in redox state [17]. Redox state has been reported to play no role in glomus cell O_2 sensing [28,55].

In direct contrast to the above, the ROS hypothesis developed by Schumacker [13,31,56] proposes that generation of ROS from complex III of the ETC increases during hypoxia in a number of cell types including cardiomyocytes, hepatocytes and PASMCs, and that this ROS acts as the signalling mediator for subsequent responses, ranging from HPV and elevation of intracellular Ca^{2+} to increased transcription of hypoxia-sensitive genes. Inhibition of the proximal ETC would prevent this rise in ROS production, and thus studies showing that such inhibition blocks the response to hypoxia without mimicking it are entirely consistent with this hypothesis [15,27,38,39], as are studies showing potentiation of HPV or the associated rise in intracellular [Ca^{2+}] following treatment with low concentrations of cyanide [15,27,38]. Furthermore, the large majority of studies

report that exogenous scavengers of ROS, application of SOD and catalase, or overexpression of catalase or glutathione peroxidase (which degrade peroxide) suppress HPV and/or the hypoxia-induced elevation in intracellular [Ca^{2+}], but importantly do not mimic hypoxia [15,38,57–59].

It is interesting to note that AMCs clearly differ from PASMCs in this respect as well as in terms of the effect of ETC inhibitors, as in AMCs antioxidants are reported to mimic hypoxia, whilst exogenous peroxide suppresses the effects of hypoxia [17]. Moreover, in AMCs application of the complex II substrate succinate reverses the depolarisation and activation induced by the complex I inhibitor rotenone, presumably by bypassing the block and restoring electron flux to complex III, thus increasing ROS formation [17]. In pulmonary artery however exactly the same procedure restores HPV [27], i.e. it has the opposite effect on O_2 sensing. The latter is an additional argument against the Redox and energy state/AMP kinase hypotheses for HPV, as it would also be expected to restore both ROS and ATP production. However, this does not rule out a role for AMP kinase in HPV, as several studies have shown that AMP kinase can be activated by ROS and oxidative stress in the absence of any change in nucleotides [e.g. [60,61,62]].

Unfortunately, limitations of available probes have made direct measurements of ROS equivocal and controversial, with both decreases [e.g. [17,36,37]] and increases [e.g. [13,39,59,63]] in ROS production reported during hypoxia, sometimes in similar preparations and using the same probes. However, a novel ratiometric, redox-sensitive fluorescence resonance energy transfer (HSP-FRET) probe has recently been used to show an increase in ROS in cultured PASMCs [59], and hypoxia is reported to cause formation of DNA base oxidation products and membrane lipid peroxidation in pulmonary artery endothelial and PASMCs [64–66], indicative of increased oxidant stress.

3. Biosynthetic O_2 sensing mechanisms

3.1. NADPH oxidases

The phagocytic NADPH oxidase (NOX2) consists of the integral membrane sub-units $gp91^{phox}$ and $p22^{phox}$ that form cytochrome b_{558}, and the cytosolic organiser sub-unit $p47^{phox}$ which translocates $p67^{phox}$ and Rac from the cytoplasm to cause activation. In NOX1 and 3 other isoforms substitute for $gp91^{phox}$ and possibly other sub-units but are mechanistically similar to NOX2; however NOX4 apparently does not require the cytosolic sub-units for activation [67]. The primary purpose of at least NOX2 is to generate superoxide from molecular O_2, using NADPH as an electron donor; the P50 is reported to be ~1.7 kPa PO_2 (~18 μM) [68]. It is to be expected therefore that hypoxia should cause a fall in NOX-derived ROS, and it has been proposed that this underlies O_2 sensing in a number of tissues including NEBs, glomus cells and PASMCs [18,69,70]. However, some studies have shown that hypoxia causes an apparently paradoxical increase in NOX activity and ROS production in PASMCs [71] and glomus cells [72]; this could be as a result of a concomitant increase in NADPH [73,74] (Fig. 3).

There is strong evidence that NOX2 and a reduction in ROS underlies the response to hypoxia in NEBs, with consequent inhibition of K^+ channels and depolarisation, an important component of this evidence being loss of O_2 sensing in $gp91^{phox}$-deficient mice [18,75,76]. In contrast, O_2 sensing was retained in glomus cells, AMCs and lung from mice lacking $gp91^{phox}$ [39,77–79], effectively ruling out any role for NOX2 in these tissues. Although knockout of $p47^{phox}$ did not abolish O_2 sensing in either lung or glomus cells, in the former it caused inhibition of a rapid transient phase of HPV [39], whilst in glomus cells it enhanced O_2 sensitivity, and abolished the hypoxia-induced elevation in ROS [72]. It has been suggested that the latter represents an important modulatory role for a non-phagocytic NOX in glomus cells [73]. Interestingly, NOX4 has been shown to confer O_2 sensitivity upon TASK-1 channels in a model cell system [80], and TASK-1 has been implicated in O_2 sensing for both PASMCs and glomus cells [34,81,82]. The P50 for activation of TASK-1 in glomus cells is reported to be 1.6 kPa PO_2 [81], and whilst I could find no value for the P50 of NOX4, this is remarkably similar to that of at least NOX2.

3.2. Heme oxygenases

Three isoforms of heme oxygenases (HO) have been identified, HO-1, -2 and -3, although only HO-2 is constitutively expressed. HO degrades heme to CO, biliverdin and Fe(II) in the presence of O_2 and NADPH [83]; it is reported to have an exceptionally high O_2 affinity, with a P50 for HO-2 of ~0.001 kPa PO_2 (0.013 μM) and for HO-1 ~0.0035 kPa PO_2 (0.036 μM) [84]. It has recently been proposed that a novel association between HO-2 and large conductance, Ca^{2+}-activated K^+ (BK) channels in the membrane underlies O_2 sensing in glomus cells, and possibly elsewhere [85,86]. This hypothesis proposes that HO-2-derived CO maintains BK channel activity in normoxia, but in hypoxia the reduction in CO formation leads to channel closure and depolarisation. Although one study in HO-$2^{-/-}$ mice has reported that the ventilatory response to hypoxia was indeed attenuated [87], a subsequent report showed no effect on glomus cell O_2 sensitivity, or indeed a role for BK channels in O_2 sensing [88]. However, these discrepancies could well reflect species differences, as part of the original study was performed in rat. It has been suggested that HO-2 may also be involved in PASMC O2 sensing, as HO-$2^{-/-}$ mice had ventilation/perfusion mismatch and were hypoxaemic [87], but there is little evidence that BK channels are involved in HPV [5], and inhibition or knockdown of HO-2 facilitates HPV [89].

3.3. Cytochrome P-450 monooxygenases

Cytochrome P-450 monooxygenases (CYP) include a vast number of homologous O_2 sensitive proteins that oxidise a wide range of compounds, although in terms of distal mediators attention tends to have focused on those metabolising arachidonic acid. Of these, ω-hydroxylases generate ω-terminal hydroxyeicosatetraenoic acids (19- and 20-HETE),

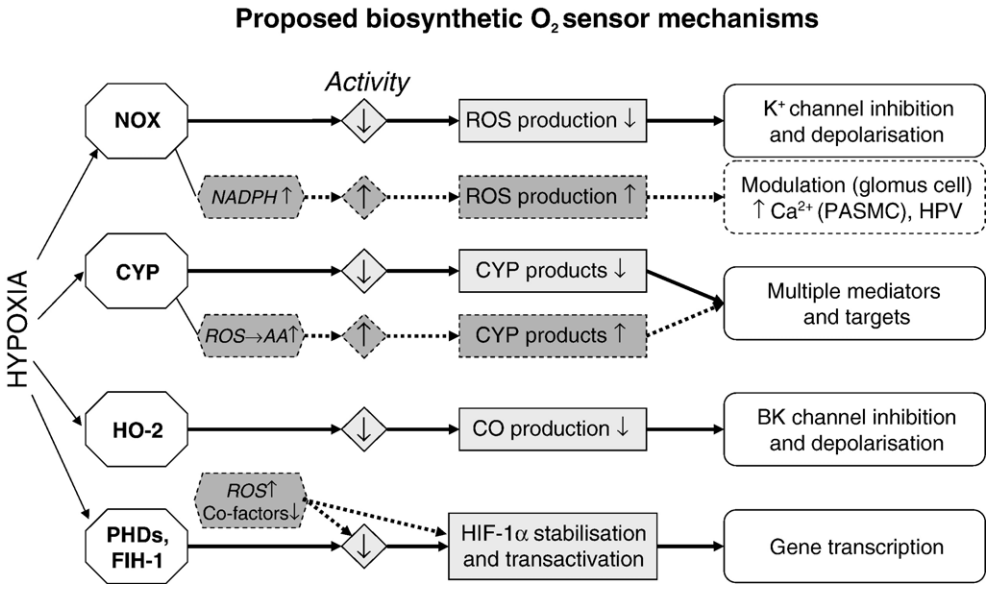

Fig. 3. Summary of biosynthetic O_2 sensor mechanisms. Boxes surrounded and connected by dashed lines represent potential interactions with bioenergetic O_2 sensor mechanisms; if these represented the major mechanisms then NOX and CYP would no longer be regarded as O_2 sensors, but as part of the effector pathway. In the case of PHDs however the bioenergetic pathway could be parallel or synergistic.

and epoxygenases generate *cis*-epoxyeicosatrienoic acids (EETs); mid-chain hydroxylation of arachidonic acid also forms HETEs, including 11-, 13- and 15-HETE [90]. The P50 for ω-hydroxylases is reported to be relatively high at ~9 kPa PO_2 (90 μM) and greater still for epoxygenases [91]. Though this suggests that $[O_2]$ should be rate limiting, certainly in the cytosol, and that a fall in PO_2 would therefore reduce synthesis of product, there is in fact evidence that arachidonic acid availability is the primary limiting factor [90]. As hypoxia and ROS both activate phospholipase A_2 and increase arachidonic acid liberation from membrane lipids [92], this could potentially mean that hypoxia stimulates rather than inhibits CYP activity, at least for some isoforms.

Numerous reports have in fact suggested that CYP metabolites contribute to the response to hypoxia in the systemic microvasculature and endothelium [90,93,94], and may contribute to HPV [95]. It has recently been proposed that a CYP 2C9 epoxygenase product plays a major role in both HPV and the vascular remodelling associated with chronic hypoxia [96], as both were suppressed by a novel inhibitor of CYP 2C9, and overexpression of CYP 2C9 increased pulmonary vascular tone. However, other CYP inhibitors that also block CYP 2C9 do not cause selective inhibition of HPV [97,98], nor do they affect O_2 sensing in glomus cells [55].

3.4. HIF-1 and prolyl and asparaginyl hydroxylases

The transcription factor hypoxia-inducible factor-1 (HIF-1) is responsible for many of the alterations in gene expression that allow adaptation to prolonged hypoxia, for example those underlying erythropoiesis [99], angiogenesis [100], and expression of ion channels [101]. HIF-1 is formed of a constitutively expressed HIF-1β sub-unit and an O_2 regulated HIF-1α sub-unit. In normoxia HIF-1α is rapidly degraded because it is ubiquitinated by a complex containing von Hippel–Lindau (vHL) protein; in hypoxia HIF-1α is stabilised because vHL binding requires O_2-dependent hydroxylation of proline residues on HIF-1α by Fe(II) and 2-oxoglutarate requiring prolyl hydroxylase domain proteins (PHD) [102]. This of course implies that HIF-1α is being constitutively synthesized; its level of expression therefore reflects the balance between rates of formation and degradation. HIF-1α activity also requires transactivation, which is prevented in normoxia by hydroxylation of an asparagine residue by the asparaginyl hydroxylase FIH-1 (factor inhibiting HIF-1) [103]. Thus hypoxia allows both stabilisation and transactivation of HIF-1α, and promotes transcription. Both PHDs and FIH-1 apparently have a particularly low affinity for O_2, with the P50 for PHDs reported to be as high as ~22 kPa PO_2 (230 μM), and for FIH-1 ~9 kPa PO_2 (90 μM) [104].

Other factors such as nitric oxide (NO) and ROS can lead to HIF-1α stabilisation [105,106], and use of ETC inhibitors or cells with dysfunctional mitochondria has led to the suggestion that during hypoxia an increase in mitochondrial-derived ROS is essential for stabilisation [107–109]. However, an alternative and experimentally convincing interpretation of such studies is that inhibition of mitochondrial function prevents HIF-1 stabilisation during hypoxia because it reduces O_2 consumption and so increases O_2 availability [62,110], though this cannot account for reports that antioxidants suppress hypoxia-induced HIF-1α stabilisation [109,111]. Interestingly, although 1 mM cyanide increased basal expression of HIF-1α in HeLa cells by ~50% in normoxia, it had little effect on the increase in expression induced by hypoxia and did not alter the P50 for HIF-1α expression [112] (and see below).

4. Matching the sensor to the sensed

4.1. Distribution of PO_2 values in the body

Delivery of O_2 to the cells is driven by the concentration gradient set up by consumption in the mitochondria (Fig. 4); O_2 is delivered by convection in the airways and blood vessels, and diffusion across the alveolar-capillary membrane and between blood and mitochondria. Measurements of PO_2 in the airways and major blood vessels are relatively easy, and biochemical studies provide reasonable estimates of cytosolic PO_2; this will vary between tissues according to rates of O_2 consumption and delivery, but is likely to be in the region of 1–2 kPa [21,22]. O_2 sensitive microelectrodes and more recently phosphorescence quenching methods have been used to provide estimates of PO_2 in the microvasculature and tissues [113]. These suggest that the median PO_2 in systemic arterioles is ~7 kPa, falling to ~3–4 kPa in precapillary arterioles and capillaries. The PO_2 gradient between capillaries and tissue interstitium appears to be small or non-existent in most tissues examined, suggesting that most O_2 exchange occurs in small arterioles rather than capillaries as originally surmised [113–115] (and Fig. 4). A significant proportion of the O_2 gradient across the arteriolar wall appears to be due to O_2 consumption by the walls themselves, specifically by the endothelium, and this may also account for part of the fall in PO_2 between large arteries and arterioles [113,115]. Notably, the trans-arteriolar wall PO_2 gradient has been reported to be reduced in endothelial NO synthase$^{-/-}$ mice, and tissue PO_2 increased [116]. An important point in terms of this review is that as the PO_2 of arterial blood falls during respiratory hypoxia, that in the arterioles and tissues falls in strict proportion [114].

The distribution of PO_2 has implications concerning the actual PO_2 seen by O_2 sensors in different locations. Consistent with the data shown above, exchange vessels in the carotid body are reported to have a PO_2 of ~3 kPa in normoxia [117]. The precise PO_2 seen by the small pulmonary resistance arteries (50–400 μm diameter) that primarily control pulmonary vascular resistance is unclear. It is however well known that HPV is related more strongly to the alveolar rather than mixed venous PO_2, supported by findings that there is considerable gas exchange between the alveoli and small pulmonary arteries [118,119]. NEBs are also likely to be exposed to a PO_2 close to that in the alveoli. It should be noted however that NEBs and AMCs are thought to have their primary roles in the fetus and during parturition, and the fetal arterial PO_2 is ~5 kPa.

4.2. Correlation of sensor O_2 sensitivity with regional PO_2

An important consideration for the following discussion is that the observed response of O_2 sensitive tissues tends to be correlated to the PO_2 of the arterial blood for experiments *in vivo*, or that of the perfusate or superfusate for experiments *in vitro*. This may significantly overestimate the true P50 of the O_2 sensor, particularly in superfused multi-cellular preparations where the diffusional distance to the core may be much greater than in a preparation perfused via its vasculature [120]. Similar considerations apply to cells in culture, especially when using standard gas-impermeable culture dishes, where the extensive diffusional distance from the gas phase to the cells (~2–5 mm) can lead to a substantial O_2 gradient even where cellular O_2 consumption is relatively low [110].

Whilst bearing the above in mind, it is still edifying to show the P50 reported for the various O_2 sensing mechanisms in the context of the PO_2 found in different regions (Fig. 4). It is immediately apparent that the reported P50s for cytochrome *aa3*, HO and PHDs fall outside the extremes of PO_2 in relevant

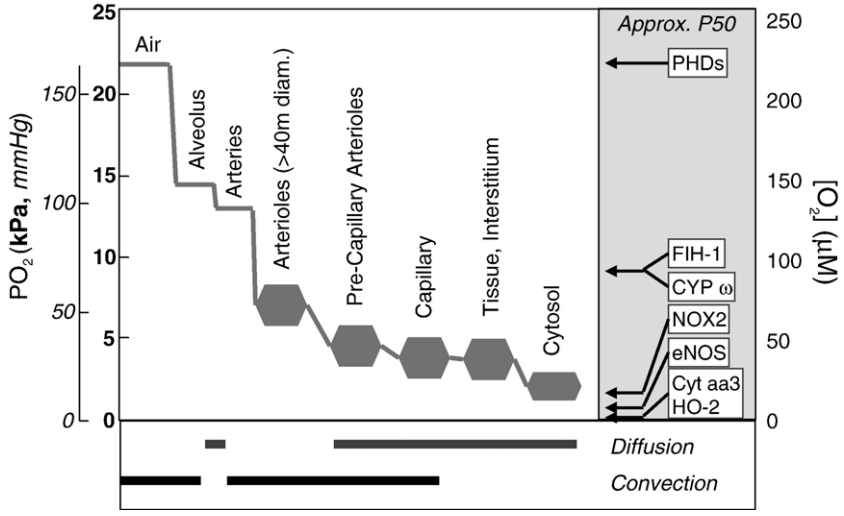

Fig. 4. Representation of the regional distribution of PO_2 from the airways to the cytosol. The thickness of the line represents variation between reports; data was derived from various sources, including [21,22,113–115,117]. The bars show the regions where O_2 transport is mediated via convection or diffusion; both are involved in small arterioles (see text). The diagram also shows the approximate values for the P50 of key O_2 sensitive mechanisms; eNOS (endothelial nitric oxide synthase) is shown for comparison. Cyt aa3, cytochrome *aa3*; CYP ω, cytochrome P-450 ω-hydroxylase; FIH-1, factor inhibiting HIF-1; HO-2, heme oxygenase 2; NOX2, NADPH oxidase, gp91^{phox} isoform; PHDs, prolyl hydroxylase domain proteins.

regions, which raises questions as to their suitability as O_2 sensors *in vivo*. However, such an examination may be misleading for a number of reasons. Firstly, the P50 of the sensor mechanism itself (rather than that of its effectors) is rarely examined *in situ*, where it may be modulated by a number of factors (see below). Secondly, it could be considered that the most important function of any O_2 sensor is to respond effectively to a change in PO_2 rather than an absolute value. For example, how much does the sensor's activity change when confronted by a 50% fall in PO_2 from normoxia, a challenge commonly used in investigations of acute and long term hypoxia? Fig. 5 shows a theoretical model based on an assumption of hyperbolic kinetics and the reported P50s of the O_2 sensing mechanisms previously discussed. Panel A shows the predicted activity of each sensor against PO_2; for comparison with Fig. 4 the approximate alveolar, arterial, tissue and cytosolic PO_2 values are shown. Note that as all of these sensors utilise O_2 their activity will fall as PO_2 is reduced, assuming other substrates and co-factors remain constant (see discussion of NOX and CYP above), although that of their distal signalling mechanisms may not (e.g. HIF-1 stabilisation, mitochondrial ROS generation). In contrast, panel B shows the predicted change in activity in response to a 50% reduction in PO_2 from a range of initial PO_2s.

Perusal of these two plots is enlightening. In particular, this admittedly simplistic model suggests that although the absolute activity of PHDs and FIH-1 would be very low in the cytosol, the relevant location for their proposed action, halving the PO_2 causes an almost 50% reduction in activity, and that this dynamic efficiency is retained over a wide range of PO_2. The fact that the absolute activity of PHDs is low is not necessarily inconsistent with the fact that there is little HIF-1 expression in normoxia [102,110], as PHDs may be sufficiently in excess to cause near complete degradation even at this low activity. A similar consideration applies to FIH-1. At the other extreme the predicted activity of COX, and particularly of HO-2, remains close to 100% as the PO_2 falls well below estimated cytosolic values, and even though the mitochondrial PO_2 might be expected to be lower than this, even a reduction in PO_2 from 0.3 to 0.15 kPa would only reduce the predicted COX activity by ~15%. This does not appear to correlate well with the reported sensitivity of O_2 sensitive tissues where inhibition of oxidative phosphorylation or HO-2 activity has been proposed as the initial event in O_2 sensing.

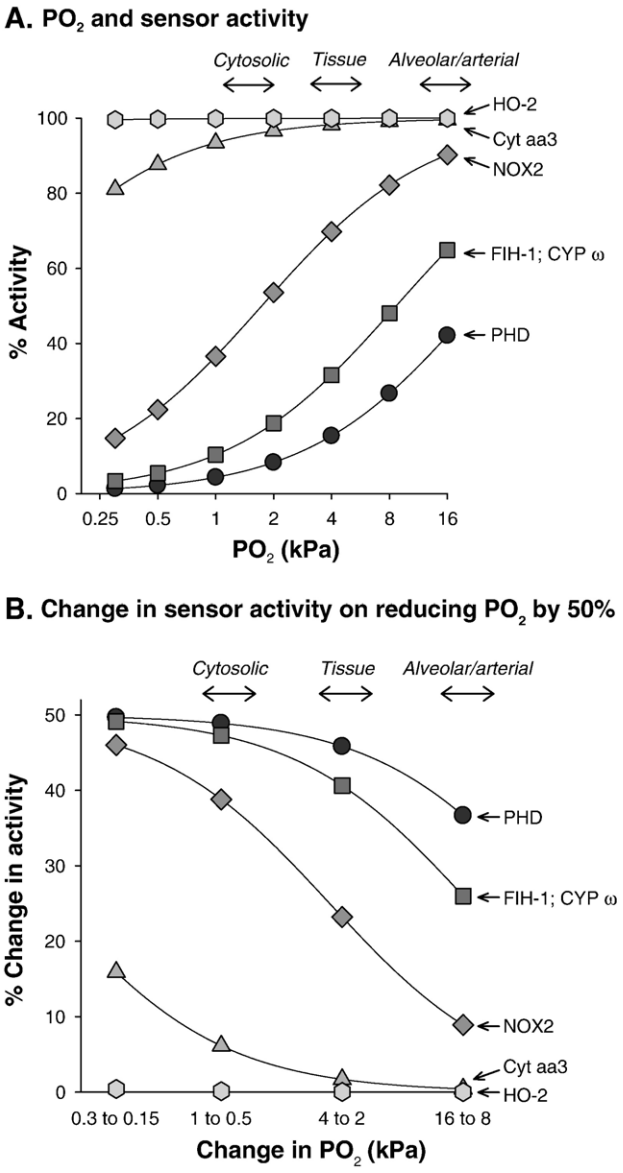

Fig. 5. Panel A: Theoretical model of the activity of proposed O_2 sensor mechanisms over a physiologically relevant range of PO_2, calculated assuming hyperbolic, Michaelis–Menten kinetics, and substrates and co-factors other than O_2 in excess (if applicable). Panel B shows the calculated change in sensor activity predicted for a halving of the PO_2 from 8, 4, 1 and 0.3 kPa, approximating to normoxic values for alveoli/arteries, tissue, cytosol, and possibly mitochondria. Values for the P50s were taken from: PHDs and FIH-1 [104]; CYP ω-hydroxylase [91]; NOX2 [68]; cytochrome *aa3* (COX) [21]; and HO-2 [84].

4.3. Correlation of sensor and effector O_2 sensitivities

Fig. 6 illustrates example responses to decreasing PO_2 for various O_2 sensitive preparations, using data derived and extrapolated from the literature. These specific preparations were chosen as they were either perfused (carotid body [121], lungs [122]), or if superfused the diffusional distances were equivalent to those in vivo (thin walled small pulmonary arteries [40,123]); for these preparations the values of PO_2 shown on the abscissa reflects that within blood vessels. The neonatal AMC preparation on the other hand consisted of superfused clusters of 5–30 freshly isolated cells [124], and the superfusate PO_2 is probably more representative of that in tissue. The HeLa cells were studied under closely controlled conditions in a tonometer, and in the presence of cyanide to block mitochondrial O_2 consumption and thus minimise the O_2 gradient across the cell membrane; the PO_2 can therefore be considered equivalent to that of the cytosol [112]. Thus the response curves for carotid body, pulmonary artery and lung would be shifted to the left by a factor of ~5–10, and that for AMCs by ~2 if the abscissa referred to cytosolic PO_2 (see Fig. 4 and [120]). Whilst these are broad approximations, it is notable that the AMC response

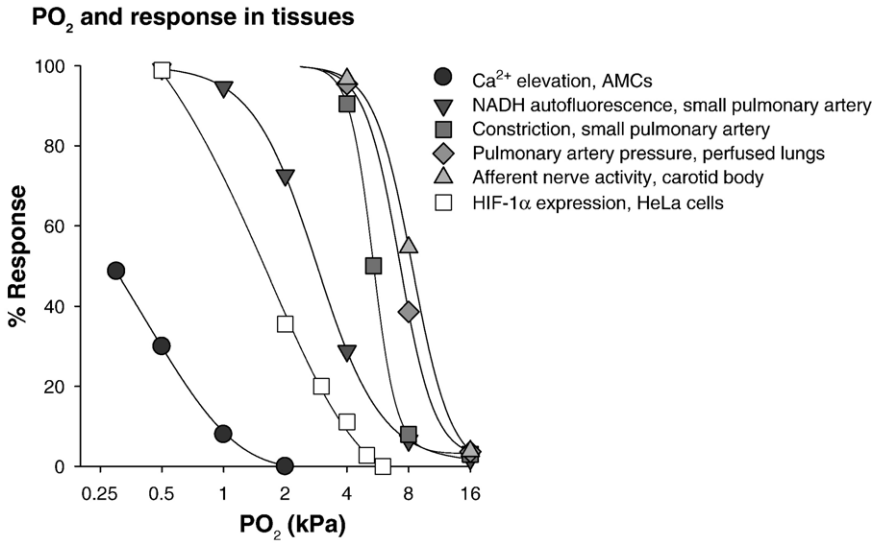

Fig. 6. Depiction of the response of some classical O_2 sensitive tissues or mechanisms to changes in PO_2. The data used for generating the figure were derived and interpolated from examples in the literature; symbols are for identification only, and do not necessarily reflect data points. The preparations and their recorded responses are: elevation of intracellular [Ca^{2+}] in superfused AMCs derived from neonatal rats, estimated using Fura-2 or Indo-1 [124]; increase in NADH autofluorescence in small intrapulmonary arteries of rat [40] (and own unpublished observations, Ward, Baxter and Aaronson); constriction of pressurised small intrapulmonary arteries of pig [123]; elevation of pulmonary artery pressure in blood-perfused lungs of pig to a step-wise reduction in PO_2 [122]; steady-state responses of single afferent nerve fibres from the carotid body of cats, perfused *in situ* [121]; Western blot estimation of HIF-1α expression in HeLa cells maintained in a tonometer at the given PO_2 for 4 h, in the presence of 1 mM cyanide to minimise O_2 consumption and gradients [112].

curve would remain to the left of the others, presumably reflecting the correspondingly lower arterial PO_2 in the fetus and at parturition [3].

The relationships between PO_2 and final response of carotid body (afferent nerve activity), pulmonary artery (constriction) and perfused lungs (increase in pulmonary artery pressure) span a physiologically appropriate range of PO_2 and are very steep (Fig. 6), suggesting the coupling function between sensor and response is highly non-linear. This is not particularly surprising considering the number and complexity of potential intermediate pathways, especially for example if AMP kinase is involved, which responds according to at least the square of the ADP/ATP ratio [41]. An important point that is often overlooked is that the greater the power of the coupling function the more the P50 of the response will move to the right of that of its sensor, though with no change in threshold. The increase in mitochondrial NADH with decreasing PO_2, as shown in Fig.6 and as estimated from autofluorescence in intact small pulmonary arteries [27,40], is probably a relatively direct reflection of inhibition of mitochondrial function (see above). Assuming that the latter is indeed the basis of O_2 sensing in PASMCs, then comparison of the NADH autofluorescence response curve in small pulmonary arteries with that for constriction in a similar preparation (Fig. 6) might be said to exemplify the effect of an amplifying coupling function, with a shift to the right of the P50 for constriction but no change in threshold. A high coupling function endows a physiological advantage as it increases sensitivity over a narrow range, a functional requirement for the control of ventilation and HPV.

The P50 of the NADH autofluorescence response shown is ~2.8 kPa PO_2 in superfusate (Fig. 6). Even if it is assumed that the cytosolic PO_2 is as little as 10% of that in the superfusate (Fig. 4) and the P50 is corrected accordingly to 0.28 kPa, this is still ~40 times the reported P50 for COX of 0.07 kPa [21]. Likewise, in glomus cells even moderate hypoxia has been shown to increase NADH autofluorescence and reduce mitochondrial flavoproteins and $\Delta\Psi m$ [10,33]. By comparison, the P50 for AMCs is ~0.3 kPa [124]; once corrected for cytosolic PO_2 this shows good correlation with the P50 of COX, as predicted if the latter is the O_2 sensor in these cells. This implies that the mitochondria of carotid body glomus cells and PASMCs must either be specialised so that they are affected by higher levels of PO_2 than in other tissues, or that these tissues contain modulating influences that effectively increase the P50.

The large divergence between the P50 reported for carotid body (~0.85 kPa, as corrected for cytosolic PO_2) [121] and that for HO-2 (0.0012 kPa) [84] is considerably more severe than that for COX, perhaps fatally so for the hypothesis that HO-2 acts as the O_2 sensor in glomus cells. Nevertheless, HO-2 dependent responses were elicited by moderate hypoxia (3–5 kPa) [85]. This implies either that the P50 for HO-2 is very much higher when it is associated with BK channels, or that the published value for the P50 is simply incorrect or species dependent, or alternatively that HO-2 does not act as the sensor itself, but via CO facilitates or permits sensing by the BK channels themselves. Only further studies will determine which if any of these options is correct.

The PO_2 response curve for HIF-1α expression deserves special mention, as in contrast to the other mechanisms discussed the P50 of the response (~1.7 kPa PO_2) is far lower than that of the sensor (PHDs; ~22 kPa PO_2), rather than higher. In addition, HIF-1α expression increases in a close to exponential

fashion as PO_2 falls towards zero [112], whereas the relationship between PHD activity and PO_2 is not far from linear over the same range (Fig. 5A).

This has been cited as evidence that the rate of HIF-1α degradation cannot be simply a function of the effect of PO_2 on PHD activity, but must instead be an integrated response, possibly involving NADPH oxidase or mitochondrial-derived ROS, and the effect of these and hypoxia on the necessary co-factors for PHD hydroxylation of HIF-1α, specifically ascorbate, Fe(II) and 2-oxoglutarate [10]. The arguments for such an integrated model are compelling, though the data shown in Fig. 6 for example were obtained following inhibition of oxidative phosphorylation with 1 mM cyanide [112]. In any case, the expression of HIF-1α cannot be expected to have a simple relationship with PO_2, as it is hydroxylated HIF-1α (which is then rapidly degraded) that is the product of the PHD-catalysed reaction, and expression of HIF-1α will depend on the balance between synthesis and degradation (see above). Moreover, the rate of hydroxylation of HIF-1α will depend not only on the concentrations of its two substrates, O_2 and HIF-1α, but also on those of its required co-factors, which are not necessarily in excess, especially in hypoxia [10]. Doubtless an appropriate kinetic model could be developed to define the relationship between PHD activity, $[O_2]$ and HIF-1α expression, but this is beyond the scope of this review (and its author). Notwithstanding the above, a notable though almost certainly serendipitous point is that reducing the cytosolic PO_2 from 2 to 1 kPa results in an approximate doubling of HIF-1α expression (Fig. 6), and an approximate halving of PHD activity (Fig. 5B).

4.4. Modulation of O_2 sensitivity of the sensor

There is no need to consider modulation of the O_2 sensitivity of PHDs and FIH-1, and probably CYP, as it has already been shown that these can be effective over a wide range of PO_2 and within their operational locus (the cytosol). As discussed above however there is a clear dichotomy between the P50s for COX and HO-2 and their proposed effectors in glomus cells and PASMCs, though not for COX in AMCs. There is little or no information available on factors that might modify the P50 for HO-2, so this will not be discussed further. However, a number of factors are known to alter the effective P50 for COX, and some that could potentially affect mitochondrial signalling in hypoxia that do not necessarily involve COX.

The first consideration is that values for the P50 for COX commonly quoted in discussions of mitochondrial O_2 sensing are derived from tissues other than the classical O_2 sensitive tissues of glomus cell and PASMC (as indeed I have done here), as these are the only ones available. However there is strong evidence that mitochondria in glomus cells and PASMCs have a lower affinity for O_2 (i.e. the P50 is greater) than in non-O_2 sensing tissues or indeed AMCs, and may also differ in terms of basal respiratory rate and $\Delta\Psi m$ [10,27,33,36,125], and a low O_2 affinity cytochrome *aa3* has been implicated in glomus cells [126,127]. The effective O_2 affinity of COX is also proportional to its relative expression compared to that of proximal components of the ETC; a low relative expression will not only increase the P50, but also make the proximal ETC more reduced and promote formation of ROS [21,22]. Interestingly, the mitochondrial P50 is increased 2–3 fold above normal in cells from patients with Leigh syndrome who suffer from mutations in SURF1, and consequently defective assembly of COX [128].

The P50 of COX can also be affected by cytosolic mediators, potentially allowing dynamic regulation. NO is a known modulator of mitochondrial function, and has been shown to compete with O_2 at cytochrome *aa3*, increasing the effective P50 and promoting ROS production [129]. It has been suggested that this mechanism allows the systemic circulation to respond to moderate hypoxia and vasodilate [130]. However, inhibition of NO synthase tends to augment both the response of glomus cells to hypoxia and HPV [16,131]. H_2S is believed to be an endogenous "gasotransmitter" with functional similarities to NO and CO; its synthesis and metabolism are highly redox dependent [132]. It has recently been suggested that H_2S may be involved in O_2 sensing in the pulmonary and systemic vasculature, based on evidence that inhibition of its synthesis suppresses the response to hypoxia, whereas exogenous H_2S mimics hypoxia [133]. Like NO, H_2S also binds to cytochrome *aa3* and competes with O_2 [134], and promotes mitochondrial ROS production [135]. However, as yet there is insufficient information to come to any form of decision as to the role of H_2S in O_2 sensing.

Finally, two other mechanisms may affect the effective P50 for mitochondrial redox state and/or ROS production, without necessarily affecting COX electron flux or ATP production. The first is the "near equilibrium" hypothesis, which has already been alluded to and predicts that cytochrome *c* and the proximal ETC become more reduced even well above the P50 for COX as the PO_2 falls [23,24]. The second, proposed by Schumacker [13,136], invokes hypoxia-induced changes in the lipid–protein structure of the mitochondrial inner membrane such that there is either i) a shift of ROS production and extrusion from the Qi to the Qo side of complex III, ii) an increase in ubisemiquinone lifetime in complex III (and hence a greater likelihood of electron transfer to O_2), or iii) increased access of O_2 to ubisemiquinone. As yet, there is no strong evidence for or against these interesting hypotheses; they do however raise the potentially important concept of a separation of mitochondrial O_2 sensing mechanisms from those of energy production.

5. Conclusion

This review has attempted to put some of the key mechanisms that have been proposed to underlie O_2 sensing into the context of the environment in which they are thought to function. This is rarely considered in discussions of O_2 sensor function, but is vital if any sense is to be made of correlations between the P50 of sensor, signalling mediators, and the ultimate response. Whilst admittedly some of the interpretations are based on assumptions that may prove to be of limited validity, I believe that these provide a reasonably accurate view of some of the strengths and weaknesses of current hypotheses of O_2 sensing in what may be regarded as their natural habitat. In particular, the high P50s for PHDs and FIH-1 should not be of concern when considering their role in O_2 sensing, as they have a sufficient dynamic

response over the appropriate range of PO_2, though co-factor limitations for PHDs at low PO_2 might conceivably accelerate HIF-1 stabilisation. In contrast the role of HO-2 as an O_2 sensor would seem to be severely limited by its (allegedly) extremely high affinity for O_2. Whilst the same criticism might be levelled at mitochondria, there is strong evidence that at least in glomus cells and PASMCs the effective O_2 affinity of COX is significantly less than in most other tissues.

The bulk of the evidence supports the concept that mitochondria act as the O_2 sensor in the classical O_2 sensing tissues of carotid body, pulmonary artery and AMCs, though not in NEBs. However, the means by which hypoxia-induced alterations in mitochondrial function are translated into a functional response clearly differ, involving changes in energy state and probably AMP kinase activity in glomus cells, a fall in ROS production in AMCs, and, I would suggest, an increase in ROS production in PASMCs. Whilst at first glance the latter two appear incompatible, consideration of the operation of the ETC and the effect of changes in coupling between proximal and distal components demonstrate that they are both entirely feasible. As previously suggested, this implies that the mitochondria of these specialised O_2 sensing tissues are adapted to purpose, and under these conditions should be regarded as signalling organelles rather than the power house of the cell.

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