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Review

Oxygen sensors in hypoxic pulmonary vasoconstriction

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Abstract

Hypoxic pulmonary vasoconstriction (HPV) is an essential mechanism adapting lung perfusion to regional ventilation. Perturbations to HPV, such as those occurring in pneumonia, acute respiratory distress syndrome and liver failure, can result in arterial hypoxemia. Under conditions of general hypoxia, HPV increases pulmonary vascular resistance and thus causes acute pulmonary hypertension. Despite intensive research, the underlying mechanisms of HPV have not been fully elucidated. Deciphering signalling pathways that result in HPV could suggest novel approaches to address a failure of HPV, as well as for the treatment of pulmonary hypertension associated with HPV. Within this context, this review focuses on current concepts in the oxygen sensing mechanisms that underlie HPV.

Keywords: Hypoxic pulmonary vasoconstriction; Oxygen; Oxygen sensing; Lung; Hypoxia

1. Introduction

Hypoxic pulmonary vasoconstriction is a physiological response of the lung to alveolar hypoxia, which redistributes pulmonary blood flow from areas of low oxygen partial pressure to areas of high oxygen availability. This mechanism thus optimises gas exchange and helps to prevent arterial hypoxemia [1,2].

Impairment of HPV under pathophysiological conditions, including acute respiratory distress syndrome [3] or hepatopulmonary syndrome [4], or during anaesthesia [5], may result in poor arterial blood oxygenation. Alternatively, global, prolonged alveolar hypoxia that occurs at high altitude, or during impairment of respiratory functions (for example, as occurs in chronic obstructive pulmonary disease (COPD), pneumonia, fibrosis, or neurological diseases) may result in pulmonary hypertension. Both persistent HPV and hypoxia-altered gene regulation may contribute to hypoxia-induced pulmonary hypertension.

Due to the opposing functions of lung and systemic vessels—one taking up, the other delivering oxygen—different responses to hypoxia have emerged. While systemic vessels of adults dilate during hypoxia, pulmonary vessels constrict. From an ontogenetic point of view, hypoxic pulmonary vasoconstriction may better be termed "normoxic pulmonary vasodilation". In utero, persistent vasoconstriction of pulmonary vessels helps prevent perfusion of non-inflated lungs. After birth, inflation of the alveoli and the concomitant increase in alveolar oxygen partial pressure leads to vasodilation and perfusion of the lung vasculature. Although the importance of HPV for pulmonary gas exchange was recognised early [6], the underlying oxygen sensing and signal transduction processes have not been clarified. Elucidation of the oxygen sensing and signal transduction mechanisms of HPV could

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serve as the basis for the development of new therapeutic approaches to treat diseases associated with a disturbance in HPV or acute pulmonary hypertension associated with global HPV. Within this context, this review focuses on current concepts of oxygen sensing mechanisms that underlie HPV (Fig. 1).

2. Characteristics of hypoxic pulmonary vasoconstriction and location of the oxygen sensor

Pulmonary artery pressure is increased during hypoxic ventilation [7,8], leading von Euler and Liljestrand to suggest that ventilation-perfusion matching was the purpose of this

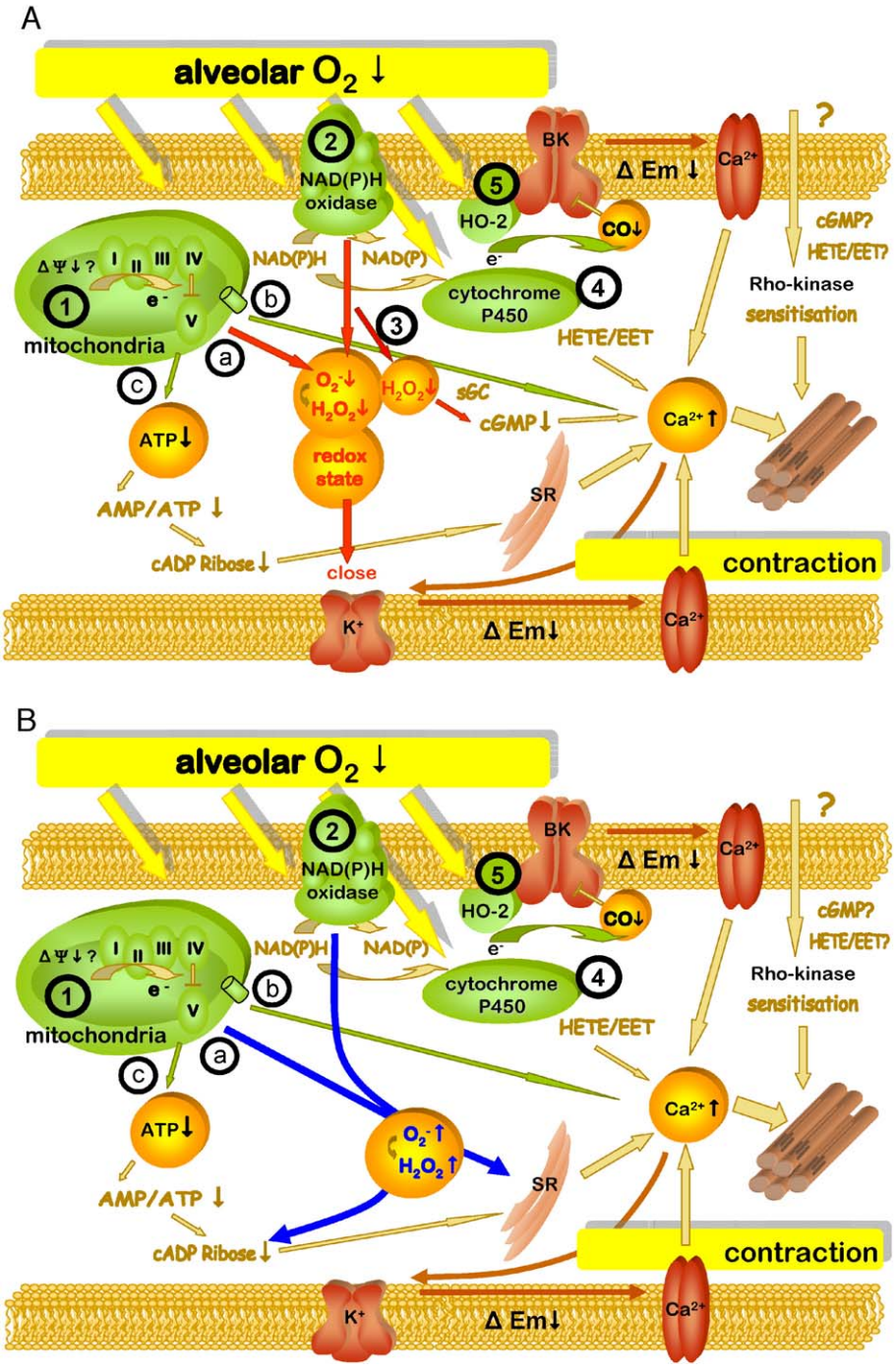

Fig. 1. Current concepts of the oxygen sensing of hypoxic pulmonary vasoconstriction. Possible oxygen sensors are shown in green, mediators of hypoxic pulmonary vasoconstriction (HPV) in yellow. For details see text. pO_2, oxygen partial pressure; $\Delta\psi$, mitochondrial membrane potential; SR, sarcoplasmic reticulum; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; ΔE_m, cellular membrane potential; BK, large conductance Ca^{2+}- and voltage-gated potassium channel; HO-2, hemoxygenase-2. (A) Includes those concepts that comprise a decrease in reactive oxygen species (ROS) as a trigger for HPV (red lines), (B) those that comprise an increase in ROS as a trigger for HPV (blue lines).

increase in pulmonary artery pressure [6]. It was believed that a self-regulatory mechanism intrinsic to the lung controls HPV, since HPV still occurs after denervation of the lung and in explanted lungs, thus excluding neural or humoral effects [9,10]. Furthermore, there were no histological or pharmacological hints of a contribution by neural mechanisms [11,12]. Along these lines, foetal pulmonary arterioles co-transplanted with neonatal lung tissue into the hamster cheek pouch demonstrated HPV before innervation [13].

The strength of the HPV response depends on species, age, gender, pCO_2, pH, and methodology employed [11,14–16], although its effector mechanism is independent from these factors. HPV is a highly conserved process in mammals [17–19], birds [20], reptiles [21], and even fish [22]. HPV is triggered by mild hypoxia (alveolar $pO_2 <$ 100 mm Hg) [18]. The precapillary smooth muscle layer of the resistance vessels, located at the acinus entrance, has been identified as the effector cell type [23–26]. Since isolated pulmonary artery smooth muscle cells (PASMC) respond to hypoxia by contraction and an elevation in intracellular Ca^{2+} levels, these cells represent both the sensor and effector cell type [27–31] in the context of acute hypoxia. For sustained (>30 min) hypoxia, a contribution of endothelial cells must also be considered [32,33].

The kinetics of sustained HPV have not been fully resolved [34–36]. There is no doubt that HPV occurs, and that HPV can be rapidly switched off, since HPV has to adapt perfusion to ventilation immediately upon changes in the alveolar oxygen partial pressure [18]. For sustained hypoxia a temporary vasodilation has been described, followed by a secondary vasoconstrictor response. Sustained HPV may be of major relevance for continuous ventilation-perfusion matching and under conditions of generalised hypoxia, which results in pulmonary hypertension.

Pathways leading to contraction of precapillary PASMC rely on an increase in cytosolic calcium, including an influx from the extracellular space as well as from intracellular stores, and membrane depolarisation attributed to closure of potassium channels [37]. The role of potassium channels in HPV is reviewed elsewhere in this *Review Focus*. For sustained HPV, a Ca^{2+} sensitisation, in addition to an increase in cytosolic calcium, possibly via activation of Rho-kinase, has been suggested [38–40]. However, it is not yet clear, if Rho-kinase and/or other protein kinases only play a modulating or an indispensible role in HPV [41].

3. The mitochondria as possible oxygen sensors of HPV

Apart from being the main site of oxygen consumption, two arguments are in favour of a role for mitochondria as primary oxygen sensors. First, inhibitors of the mitochondrial electron transport chain (ETC) specifically inhibit HPV [31,42,43], and second, PASMCs without a functional respiratory chain do not show hypoxia-specific responses [29].

In general, mitochondria have diverse functions in the cell related to energy conservation, apoptosis, calcium regulation and intracellular signalling [44]. Mitochondria generate a proton gradient across the mitochondrial membrane, thereby providing energy for ATP synthesis. The electrons are transferred along a redox gradient, and finally to molecular oxygen. The ETC consists of several complexes: complexes I and II, which provide the electrons; complex III including an electron cycle; and complex IV, the final centre for the reduction of oxygen.

Electrons from the ETC may be "accidentally" transferred to molecular oxygen, resulting in the generation of superoxide radicals. After conversion by superoxide dismutase (SOD), the resulting H_2O_2 can readily diffuse through the membrane into the cytosol. Alternatively, superoxide can pass through an anion channel from the intermembrane space into the extramitochondrial environment [43,45,46]. The putative role of mitochondria during HPV is depicted in Fig. 1, ①.

Two main theories exist concerning a role for mitochondria and reactive oxygen species (ROS) in HPV. (1) The original redox hypothesis, proposed by Weir, Archer and colleagues, assumed a decrease in mitochondrial ROS, shifting the cellular redox state towards a more reduced state, resulting in the inhibition of K_v channels (Fig. 1A, ①a). This closure of potassium channels is mediated by the redox pairs GSH/GSSG and NADH/NAD [36,47]. (2) In contrast, Schumacker, Chandel and co-workers suggested that an increase in ROS production during hypoxia triggers intracellular calcium release and thus HPV [43,48] (Fig. 1B, ①a). The latter theory assumes that mitochondrial complex III is the ROS producing site.

3.1. Mitochondria-dependent decrease of ROS in HPV

Early investigations found that the mitochondrial inhibitors rotenone, antimycin A, azide, and cyanide, as well as dinitrophenol, increased vascular pressure under normoxic conditions and subsequently inhibited HPV in isolated blood-perfused rat lungs [49]. Later, Archer, Weir and colleagues demonstrated that hypoxia and the proximal ETC inhibitors, rotenone and antimycin A, decreased lung ROS release (detected by chemiluminescence), whereas distal inhibition with cyanide increased ROS release during normoxia. Under normoxic conditions, rotenone and antimycin A increased pulmonary artery pressure and inhibited HPV, while cyanide increased vascular pressure but did not decrease HPV [50]. These findings are in line with the observation that rotenone and antimycin A mimicked HPV in isolated pulmonary arteries and in PASMCs, and decreased ROS production, concomitant with inhibition of potassium channels in PASMCs [31]. In contrast, these agents had opposing effects in renal tissue. These differences were attributed to a higher basal ROS production in the lung pulmonary arteries, compared to renal arteries, due to the lower respiration rates in lung mitochondria, the

reduced complexes I and III content, and a lower membrane potential in pulmonary compared with renal arteries [31]. Higher ROS production has also been observed in patients with complex I deficiency [51], perhaps explaining the high ROS production by mitochondria of PASMCs under normoxic conditions, compared to studies that provide evidence that there is low or no ROS release by intact mitochondria at all [52,53]. Archer and colleagues concluded from these pharmacological interventions that ETC-inhibition proximal to the site of mitochondrial ROS release at complex I or III attenuated the reduction potential of this complex, reduced ROS production, and increased pulmonary arterial pressure. Blockade of the electron mitochondrial respiratory chain distal to complex III had no effect on pulmonary vascular tone [54]. Thus, the decreased ROS release under hypoxic conditions triggers HPV via K^+-channels as explained above (Fig. 1A, ①a).

3.2. Mitochondria-dependent increase of ROS in HPV

Although a decrease in ROS production during hypoxia could be explained by decreased availability of oxygen, a prerequisite for increased mitochondria-derived ROS during hypoxia indicate an alteration in the properties of the ETC, which could be achieved by hypoxia-induced inhibition of cytochrome *c* oxidase and electron flow as initially suggested after investigations in hepatocytes [55]. Paul Schumacker's group later provided evidence that increased ROS release from the semiubiquinone binding site in mitochondrial complex III occurs under hypoxia because (1) in isolated rat lungs the proximal ETC-inhibitors rotenone, DPI and myxothiazol inhibited HPV, while the distal inhibitors antimycin A and cyanide did not [43]; (2) proximal inhibitors also attenuated hypoxia-induced contraction and increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) of PASMCs [29]; (3) catalase overexpression inhibited the hypoxia-induced $[Ca^{2+}]_i$ increase, as well as the hypoxia-induced increase in ROS [29,43]; and (4) myxothiazol attenuated hypoxia-induced increase in ROS, abolished HPV, and blocked hypoxia-induced $[Ca^{2+}]_i$ increase; but (5) antimycin A had no specific effects on the hypoxia-induced responses in isolated lungs or PASMCs [29,43]. In line with these observations, inhibition of the hypoxia-induced elevation in $[Ca^{2+}]_i$ by rotenone could be reversed by succinate in isolated pulmonary arteries of the rat [56].

Data from our laboratory obtained in isolated perfused rabbit lungs are consistent with the effect of the proximal electron chain inhibitors (inhibiting HPV without being hypoxia mimics) and the complex III inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) (mimicking HPV), but disagree with respect to the effects of antimycin A, inhibiting HPV without being a hypoxia mimic and cyanide, inhibiting HPV [42,57]. Complex II of the ETC has also been suggested to be a source of ROS release in HPV in a study in murine lung sections [58]. While ROS production under normoxic conditions required complexes I and III in this investigation, ROS generation under hypoxic conditions also required complex II. Inhibition of the reversed enzymatic reaction of the succinate dehydrogenase, i.e., fumarate reductase, by application of succinate, specifically abolished ROS generation under hypoxic, but not normoxic, conditions [58].

While these studies substantially relied on the use of inhibitors, PASMCs that lack a functional ETC were incapable of generating ROS under hypoxic conditions, and lost their response to hypoxia, although they still responded to the thromboxane mimetic U46619 [43,59].

It has been suggested that ROS can escape from the mitochondria through an anion channel, and subsequently induce vasoconstriction by downstream effects on Ca^{2+} metabolism and sensitivity (Fig. 1B, ①a). Alternatively, these channels are dependent on mitochondrial membrane potential [46]. This may in turn be affected by respiration, calcium metabolism and mitochondrial ATP-sensitive potassium channels, that may also affect mitochondrial ROS release and thus again interact with HPV [60], suggesting an even more complex role in HPV than simple ROS trafficking. The current data do not support a conclusive role for mitochondria in HPV, particularly concerning their contribution to ROS release. The diverging effects of different mitochondrial inhibitors in various investigations may be explained by the different experimental settings, species, tissues and concentrations of the inhibitors used, as well as the methods used for quantification of ROS. They may also be attributed to properties of these agents exceeding simple variation of ROS production, for example, also affecting calcium homeostasis and ATP production [45,61].

3.3. Mitochondria and calcium homeostasis

Mitochondria play a role in cytosolic calcium homeostasis through a calcium uniporter that is driven by the mitochondrial membrane potential, and the concentration of cytosolic calcium [45]. A decrease in membrane potential due to impaired respiration induces mitochondrial calcium release, and the intracellular calcium profile can be shaped by mitochondrial buffering of changes in $[Ca_{2+}]_i$ [44].

In carotid body cells, hypoxia <60 mm Hg leads to mitochondrial membrane depolarisation [62], which could reduce mitochondrial Ca^{2+} uptake, resulting in an increase in cytosolic calcium (Fig. 1, ①b). Cyanide, rotenone and uncouplers like carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) increase $[Ca^{2+}]_i$ in PASMCs during calcium release from the sarcoplasmic reticulum [63–65]. It was therefore suggested that inhibition of mitochondrial calcium uptake by FCCP or hypoxia augmented intracellular calcium increase [66]. Furthermore, an increase in hypoxic tone and HPV in isolated lungs has been described using mitochondrial uncouplers [18]. Thus, mitochondria

may play an as-yet-unproven role in HPV related to Ca^{2+}-homeostasis independent of or in addition to mitochondrial ROS release.

3.4. Mitochondrial ATP production, energy state, and cADP-ribose

The role of ATP as a second messenger for HPV has been suggested since oxidative phosphorylation is the main oxygen consumption site, and inhibitors of the ETC and of glycolysis caused vasoconstriction [67,68]. However, the role of ATP was questioned because (1) changes in the ATP content or deterioration of energy state during acute hypoxia could not be detected [69–71], (2) the K_m for cytochrome *c* oxidase seemed to be too low to decrease ATP under conditions of mild hypoxia [72], and (3) inhibitors of the cytochrome *c* oxidase induced vasoconstriction under normoxic conditions, but did not abolish HPV in some investigations [43]. In contrast oxidative ATP generation may be impaired and the cellular energy state is maintained by upregulation of glycolysis during sustained HPV [56,69,70]. For example, in isolated ferret lungs, Wiener and colleagues showed that high glucose levels prevented vasodilation following acute HPV, while pyruvate did not [73,74], suggesting that glucose metabolism beyond pyruvate is responsible for the inhibition of sustained HPV. Similarly, low glucose levels suppressed sustained HPV in isolated rat small pulmonary arteries. Since pyruvate did not reverse suppression of sustained HPV in this study, glucose may facilitate sustained HPV by a mechanism independent from glucose metabolism downstream of pyruvate [56]. However, the glucose concentration applied was much lower than that used by Wiener and colleagues, perhaps explaining these different results [74].

A recent new concept assumes that mild hypoxia leads to inhibition of the respiratory chain and a small decrease in ATP production, which does not affect energy state, but rather acts as a second messenger (Fig. 1, ①c). Thus, an increase in the AMP/ATP ratio activates AMP-activated protein kinase α1 (AMPK) and increases cyclic ADP-ribose (cADPR) that releases calcium through ryanodine-sensitive calcium stores as a first step in the HPV signalling mechanism [75]. This is an extension of the oxygen sensing mechanism proposed some years ago, assuming that during sustained HPV, hypoxia increases β-NADH levels, which then increase the net amount of cADPR synthesised from β-NAD+ by ADP-ribosyl cyclase, and simultaneously inhibit cADPR degradation by cADP-ribosyl hydrolase [76]. A decrease in ATP levels under mild hypoxia may be assisted by a low oxygen affinity cytochrome *c* oxidase in PASMCs, as has been proposed for carotid body cells [77,78]. Alternatively, the cADP-ribose system may also be regulated by interference with ROS, since low levels of superoxide stimulated calcium release via cADPR [79]. That this possible link to ROS plays a role in HPV, however, remains to be proven.

4. NAD(P)H-oxidase as a possible oxygen sensor of HPV

NAD(P)H-oxidases are superoxide-generating enzymes. Classical leukocyte NADPH-oxidase is a multiprotein complex, consisting of membrane-bound $gp91^{phox}$ (now also termed NOX2) and p22, which comprise the cytochrome *b*558; and cytosolic $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$. Superoxide production by NADPH-oxidase is induced by assembly of these two sets of subunits. Activation can be induced by at least a phosphorylation of $p47^{phox}$ and Rac GTPase activation [80]. A variety of NADPH-oxidase isoforms have been identified that can substitute for NOX2 (e.g. NOX1, NOX3, NOX4, and DUOX). These isoforms have unique features, including the release of superoxide into the intracellular milieu, rather than extracellularly, and produce lower amounts of superoxide than the phagocytic type [81,82]. Two isoforms of $p47^{phox}$ and $p67^{phox}$, termed NOXO1 and NOXA1 interact with NOX1 to generate high amounts of superoxide without activation by protein kinase C-dependent phosphorylation [83,84]. Regulation of NADPH-oxidase activity may involve phospholipase A2 and protein kinase C [85,86]. NAD(P)H-oxidases might also be activated by depolarisation of the cell [87] or lead to depolarisation itself [88].

The concept of NAD(P)H-oxidases as oxygen sensors for HPV (Fig. 1, ②) emerged against (1) the background that they are oxygen sensing candidates in other oxygen sensor systems [89] and (2) the study of Thomas et al. [90] which demonstrated that the NADPH-oxidase inhibitor DPI inhibited HPV. We have confirmed these data, and excluded interference with NO as a second target of DPI [91]. The NAD(P)H-oxidase concept got a second impetus after the investigations of Marshall et al. [92] and Wolin et al. [93]. The former group suggested an NADPH-oxidase related increase in superoxide as the mechanism underlying HPV, while the latter group suggested that an NADH oxidoreductase-related decrease in superoxide and H_2O_2, through stimulation of the soluble guanylate cyclase, could decrease vascular tone under normoxic conditions. This hypothesis thus suggested a “loss of normoxic vasodilation” during HPV triggered by this pathway.

Thus, two diverging concepts regarding the contribution of NAD(P)H-oxidase-derived superoxide currently exist: one proposing an upregulation (Fig. 1B, ②) and the second a downregulation of superoxide (Fig. 1A, ②).

4.1. NAD(P)H-oxidase-dependent increase in ROS in HPV

In an elegant study, Marshall and colleagues described an NAD(P)H-oxidase in pulmonary arteries with an unusually low redox potential. Isolated smooth muscle cells from small pulmonary arteries demonstrated an increase in superoxide production that was derived from an NAD(P)H-oxidase with an unusually low redox potential [92]. An upregulation of superoxide, and subsequently H_2O_2, as the underlying pathway of HPV has also been

suggested by data from our laboratory [94]. However, the major drawback of studies suggesting an NAD(P)H-oxidase as a pulmonary oxygen sensor was that they relied primarily on one NAD(P)H-oxidase inhibitor, DPI, which also inhibits other FAD-dependent enzymes, the mitochondrial ETC, as well as potassium channels [95,96]. Therefore, different NAD(P)H oxidase inhibitors were investigated: apocynin, which, however, interfered with vascular tone in general in isolated rabbit lung studies [91], and 4-(2-aminoethyl)benzenesulfonyl fluoride, which selectively inhibited HPV in isolated rabbit lungs, but not vasoconstriction induced by other mechanisms [97]. This study also suggested an NAD(P)H-oxidase-derived increase in ROS as the underlying mechanism of HPV [97,98]. In line with these studies, protein kinase C (PKC), a possible activator of the NADPH-oxidase, has been suggested to regulate HPV via NADPH-oxidases [86] (although PKC may also affect HPV without interaction with an NADPH-oxidase [41]), and a phospholipase A_2 knockout mouse exhibited reduced HPV that may also interfere with the NADPH-oxidase pathway [85,99]. This theory was confounded by the observation that $gp91^{phox}$-deficient mice fully responded to acute hypoxia [100]. Nevertheless, these experiments cannot rule out an NAD(P)H oxidase isoform being active as an oxygen sensor. In line with this hypothesis, we recently demonstrated that mice deficient in the cytosolic NADPH-oxidase subunit p47 exhibited ~25% reduced acute, but unchanged sustained HPV. This supported the concept that isoforms of the leukocyte NADPH-oxidase may, at least in part, function as oxygen sensors in HPV. That NADPH-oxidases in principle are involved in hypoxic signalling pathways was shown in studies on neuroepithelial bodies (NEB) [89].

4.2. NAD(P)H-oxidase dependent decrease of ROS in HPV

The group of Wolin and Burke-Wolin suggested that an NADH oxidase-mediated decrease in superoxide, and subsequently H_2O_2, under hypoxic conditions may lead to decreased GMP levels, through reduced stimulation of the soluble guanylate cyclase (sGC), and thus vasoconstriction [93,101]. In addition, NO may act synergistically in this pathway with respect to HPV. In principle, cGMP release from sGC can be triggered by H_2O_2, CO and NO. The release of NO in the lung is dependent on oxygen, as it is reduced under hypoxic conditions [102,103]. However, the H_2O_2-sGC concept was challenged by data that demonstrated that only NO-triggered sGC stimulation interferes specifically with HPV [104]. Recently Wolin et al. have put forward an interesting new version of their oxygen-sensing concept. According to this hypothesis, the concentration of NADPH, and therefore ROS production, is higher in pulmonary vessel cells (compared to coronary smooth muscle cells) due to higher levels of glucose-6-phosphate-dehydrogenase, the rate-limiting enzyme of pentose phosphate metabolism by the pentose phosphate pathway (PPP) [105]. Under hypoxic conditions, high levels of glucose-6-phosphate-dehydrogenase compete with glycolysis, maintain high NADPH levels, and therefore–in combination with hypoxic inhibition of NAD(P)H-oxidases–maintain high reduction levels in pulmonary cells. In contrast, in coronary smooth muscle cells NADPH is oxidised because of an inhibited PPP [101]. Thus, inhibition of PPP decreases HPV via activation of the cGMP pathway [106]. While the decrease in ROS formation by NAD(P)H-oxidases can be explained by a lack of the substrate oxygen, it remains unclear how NAD(P)H-oxidases may increase superoxide release under hypoxic conditions. The concept of an NAD(P)H-oxidase-derived increase in ROS has to assume that oxygen is not the rate limiting factor in ROS production by NAD(P)H-oxidases, but rather that the activity of the enzyme is regulated by other mechanisms, for example, an increased electron flux through the oxidase under hypoxic conditions. We have recently published data that suggest that NADPH-oxidase-derived lung superoxide release can be increased during hypoxia [107]. However, molecular proof of such mechanism is still lacking.

5. The role of reactive oxygen species (ROS) in HPV

ROS play a key role in HPV signalling, however, there is no consensus regarding the question of whether ROS are increased or decreased under hypoxic conditions (for review see [108,109]) (Fig. 1A and B). This disagreement has implications for the interpretation of the mitochondrial and NAD(P)H-oxidase concepts of oxygen sensing, therefore, we will briefly summarise here the main investigations that have focused on oxygen-dependent ROS release in the pulmonary system.

Direct measurement of ROS in isolated rat lungs revealed a decrease in superoxide under hypoxic conditions using luminol and lucigenin enhanced chemiluminescence [50,110]. These results are consistent with (a) decreased intravascular superoxide release in [107], and (b) decreased H_2O_2 levels exhaled from [111] isolated rabbit lungs undergoing hypoxic ventilation. Furthermore, ROS detection with three different dyes (amplex red, 2′,7′-dichlorofluorescin diacetate (DCFH), and lucigenin) suggested decreased ROS levels in rat pulmonary arteries maintained under hypoxic conditions [31]. Intravascular superoxide release in isolated rabbit lungs quantified with a spin probe in electron spin resonance spectroscopy (ESR) indicated a decrease in superoxide release under hypoxic conditions, however, with a tendency towards a smaller decrease in severe hypoxia [107]. In contrast, measurements in isolated porcine pulmonary arteries suggested a hypoxia-mediated increase in ROS using lucigenin chemiluminescence and DCFH, and these observations are supported by ESR spectroscopy measurements indicating release of hydroxyl and alkyl radicals during hypoxia [30]. Along these lines, cellular measurement of ROS release suggested a hypoxia-mediated increase in calf [92], rat [43,112] and rabbit

PASMCs [58]. These results were recently confirmed by a new, elegant technique, quantifying ROS with a fluorescence resonance energy transfer (FRET) sensor technique, supporting the concept of increased ROS generation [113]. We have also recently demonstrated an increase in intravascular superoxide release during hypoxic ventilation in $p47^{phox}$-deficient mice, suggesting a non-phagocytic source of increased ROS release during HPV, that is camouflaged by an overall decreased phagocytic ROS release under hypoxic conditions [57].

Although it has been suggested that limitations in the methods used for ROS detection are responsible for the discordant effects observed, it also remains possible that alternative explanations may exist. While fluorescent probes have been criticised for yielding for false-positive results, for example, as a consequence of redox cycling, the ESR techniques may also have some limitation, since they indirectly quantify ROS generation. Convincing data have been generated with the new FRET sensor technique [113]. Nevertheless this investigation could also not explain discrepancies observed by Michelakis et al. [31], using three different methods that compared the pulmonary and the renal system, which were by themselves conclusive.

Apart from artefacts related to the ROS detection methodology, the experimental models (isolated lungs, vessels, or cells) employed and the kinetics and timeframe of the measurements must also be taken into account as potential sources of error. Similarly, the question as to the location of the ROS must also be considered. For example, is it intravascular or exhaled ROS release that is representative of, or participates in, the signalling that underlies HPV, which is suggested to occur within the vascular smooth muscle cell? Therefore, the apparently conflicting conclusions concerning ROS in HPV may be explained by the hypothesis that a local, subcellular and compartmentalised regulation of ROS triggers HPV, and that this signal is obscured in the background of a general decrease in ROS production in the remainder of the cell that is not linked to HPV.

6. Cytochrome *P*450 enzymes and heme oxygenase-2 as possible oxygen sensors of HPV

Arachidonic acid (AA)-associated pathways activated by cyclooxygenase and lipoxygenase are well known potent modulators of vessel tone, but have not been shown to mediate any HPV-specific reaction [14,114]. However, the third group of metabolites derived from AA by cytochrome *P*450 monooxygenases, namely hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs), are suggested to be involved in HPV [115–117], since oxygen serves as a substrate for these enzymes (Fig. 1, ④). Cytochrome *P*450 enzymes are implicated in a new oxygen-sensing concept: in addition to heme proteins, hemoxygenase-2 (HO-2) may play a role in oxygen-sensing via cytochrome *P*450-dependent release of CO [118]. A large conductance calcium and voltage dependent potassium channel (BK (Ca)) is tightly associated with HO-2 and is activated by HO-2-derived CO under normoxic conditions [119,120] (Fig. 1, ⑤). Carotid body cells demonstrated an HO-2-dependent hypoxic BK channel inhibition, which indicated a possible role of HO-2 as an oxygen sensor that controls channel activity during oxygen deprivation. However, this elegant and new concept of oxygen sensing still needs to be proven in the pulmonary system.

7. Concluding remarks

Although intensive research concerning the mechanism of HPV started 60 years ago with the recognition of its importance for pulmonary gas exchange, the current review demonstrates that we are far from being able to draw a conclusive picture of its regulation, even at the initial step: the oxygen sensing process. Currently, more than five different concepts are discussed, some of them suggesting completely opposing mechanisms. One major aspect of this discussion is the fact that some investigations found an increase, while others a decrease in ROS generation under hypoxic conditions, which are proposed to be involved in the downstream signalling of the oxygen sensing process. While this was initially attributed to the different techniques used for ROS detection, and criticised as not being reliable, recent new techniques like ESR spectroscopy and FRET sensor technologies may help to overcome these problems, although these techniques are also unlikely to be free of methodological problems. These conflicting results may also reflect the different models employed (e.g. cellular, vessel, intact organ, intact animal investigations), and the duration of the hypoxic treatment applied. Furthermore, a localised subcellular increase in ROS may trigger HPV, but this localised effect may be covered by a decrease in ROS in the remainder of the cell, which may not be involved in the HPV pathway. Also, it has to be taken into account that the different sensors suggested may affect each other, for example, proper mitochondrial function is a prerequisite for NAD(P)H-oxidase systems to be operative.

More provocatively, one may also hypothesise that changes in ROS levels occur as a consequence of the alteration of the cellular oxygen partial pressure but are not directly linked to HPV [121], although no convincing evidence for such a hypothesis has been provided.

Although not discussed in detail in this review, different signal transduction processes, and possibly oxygen sensing processes, are involved in the regulation of the very acute phase of HPV (occurring within seconds) and the sustained phase (several hours) of hypoxia. The latter is proposed to result in hypoxia-induced pulmonary hypertension. In this regard it was shown that ATP, oxidative phosphorylation, Ca^{2+} metabolism, NAD(P)H-oxidase and mitochondria play different roles in acute and sustained HPV. Bearing this in

mind, we have to take into account that HPV is a multifactorial process [35]. In this regard, we have recently suggested that both a mitochondrial and an NAD(P)H-oxidase mechanism contribute to the regulation of acute HPV [57].

Identification of the pulmonary oxygen sensing processes underlying HPV remains a tremendous challenge, even 60 years after von Euler and Liljestrand's initial observations. However, elucidation of the molecular mechanism(s) that regulate this process would be a key step in the development of novel approaches to address an impaired HPV response, and for the treatment of HPV-related diseases. To reach this goal, new molecular tools and subcellular approaches will have to be developed and refined.

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References

[1] Marshall BE, Marshall C, Benumof J, Saidman LJ. Hypoxic pulmonary vasoconstriction in dogs: effects of lung segment size and oxygen tension. J Appl Physiol 1981;51:1543–51.
[2] Orchard CH, Sanchez de Leon R, Sykes MK. The relationship between hypoxic pulmonary vasoconstriction and arterial oxygen tension in the intact dog. J Physiol 1983;338:61–74.
[3] Naeije R, Brimioulle S. Physiology in medicine: importance of hypoxic pulmonary vasoconstriction in maintaining arterial oxygenation during acute respiratory failure. Crit Care 2001;5:67–71.
[4] Carter EP, Hartsfield CL, Miyazono M, Jakkula M, Morris KG Jr, McMurtry IF. Regulation of heme oxygenase-1 by nitric oxide during hepatopulmonary syndrome. Am J Physiol Lung Cell Mol Physiol 2002;283:346–53.
[5] Marshall BE. Hypoxic pulmonary vasoconstriction. Acta Anaesthesiol Scand Suppl 1990;94:37–41.
[6] von Euler US, Liljestrand G. Observations on the pulmonary arterial blood pressure in the cat. Acta Physiol Scand 1946;12:301–20.
[7] Plumier L. La circulation pulmonaire chez le chien. Arch Int Physiol 1904;1:176–213.
[8] Bradford JR, Dean HP. The pulmonary circulation. J Physiol (Lond) 1894;16:34–96.
[9] Robin ED, Theodore J, Burke CM, Oesterle SN, Fowler MB, Jamieson SW, et al. Hypoxic pulmonary vasoconstriction persists in the human transplanted lung. Clin Sci (Lond) 1987;72:283–7.
[10] Peake MD, Harabin AL, Brennan NJ, Sylvester JT. Steady-state vascular responses to graded hypoxia in isolated lungs of five species. J Appl Physiol 1981;51:1214–9.
[11] Fishman AP. Hypoxia on the pulmonary circulation. How and where it acts. Circ Res 1976;38:221–31.
[12] Cutaia M, Rounds S. Hypoxic pulmonary vasoconstriction. Physiologic significance, mechanism, and clinical relevance. Chest 1990;97:706–18.
[13] Davis MJ, Joyner WL, Gilmore JP. Microvascular pressure distribution and responses of pulmonary allografts and cheek pouch arterioles in the hamster to oxygen. Circ Res 1981;49:125–32.
[14] Voelkel NF. Mechanisms of hypoxic pulmonary vasoconstriction. Am Rev Respir Dis 1986;133:1186–95.
[15] Sylvester JT, Gottlieb JE, Rock P, Wetzel RC. Acute hypoxic responses. In: Bergofsky EF, editor. Abnormal pulmonary circulation. New York: Churchill-Livingstone; 1986. p. 127–65.
[16] Dumas JP, Bardou M, Goirand F, Dumas M. Hypoxic pulmonary vasoconstriction. Gen Pharmacol 1999;33:289–97.
[17] Weissmann N, Akkayagil E, Quanz K, Schermuly RT, Ghofrani HA, Fink L, et al. Basic features of hypoxic pulmonary vasoconstriction in mice. Respir Physiol Neurobiol 2004;139:191–202.
[18] Weissmann N, Grimminger F, Walmrath D, Seeger W. Hypoxic vasoconstriction in buffer-perfused rabbit lungs. Respir Physiol 1995;100:159–69.
[19] Sylvester JT, Harabin AL, Peake MD, Frank RS. Vasodilator and constrictor responses to hypoxia in isolated pig lungs. J Appl Physiol 1980;49:820–5.
[20] Fedde MR. Relationship of structure and function of the avian respiratory system to disease susceptibility. Poult Sci 1998;77:1130–8.
[21] Skovgaard N, Abe AS, Andrade DV, Wang T. Hypoxic pulmonary vasoconstriction in reptiles: a comparative study of four species with different lung structures and pulmonary blood pressures. Am J Physiol Regul Integr Comp Physiol 2005;289:1280–8.
[22] Olson KR, Russell MJ, Forster ME. Hypoxic vasoconstriction of cyclostome systemic vessels: the antecedent of hypoxic pulmonary vasoconstriction? Am J Physiol Regul Integr Comp Physiol 2001;280:198–206.
[23] Hillier SC, Graham JA, Hanger CC, Godbey PS, Glenny RW, Wagner WW Jr. Hypoxic vasoconstriction in pulmonary arterioles and venules. J Appl Physiol 1997;82:1084–90.
[24] Staub NC. Site of hypoxic pulmonary vasoconstriction. Chest 1985;88:240S–5S.
[25] Kato M, Staub NC. Response of small pulmonary arteries to unilobar hypoxia and hypercapnia. Circ Res 1966;19:426–40.
[26] Dawson CA, Grimm DJ, Linehan JH. Influence of hypoxia on the longitudinal distribution of pulmonary vascular resistance. J Appl Physiol 1978;44:493–8.
[27] Murray TR, Chen L, Marshall BE, Macarak EJ. Hypoxic contraction of cultured pulmonary vascular smooth muscle cells. Am J Respir Cell Mol Biol 1990;3:457–65.
[28] Madden JA, Vadula MS, Kurup VP. Effects of hypoxia and other vasoactive agents on pulmonary and cerebral artery smooth muscle cells. Am J Physiol 1992;263:384–93.
[29] Waypa GB, Marks JD, Mack MM, Boriboun C, Mungai PT, Schumacker PT. Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes. Circ Res 2002;91:719–26.
[30] Liu JQ, Sham JS, Shimoda LA, Kuppusamy P, Sylvester JT. Hypoxic constriction and reactive oxygen species in porcine distal pulmonary arteries. Am J Physiol Lung Cell Mol Physiol 2003;285:322–33.
[31] Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, et al. Diversity in mitochondrial function explains differences in vascular oxygen sensing. Circ Res 2002;90:1307–15.
[32] Liu Q, Sham JS, Shimoda LA, Sylvester JT. Hypoxic constriction of porcine distal pulmonary arteries: endothelium and endothelin dependence. Am J Physiol Lung Cell Mol Physiol 2001;280:856–65.
[33] Aaronson PI, Robertson TP, Ward JP. Endothelium-derived mediators and hypoxic pulmonary vasoconstriction. Respir Physiol Neurobiol 2002;132:107–20.
[34] Aaronson PI, Robertson TP, Knock GA, Becker S, Lewis TH, Snetkov V, et al. Hypoxic pulmonary vasoconstriction: mechanisms and controversies. J Physiol 2006;570:53–8.
[35] Weissmann N, Grimminger F, Olschewski A, Seeger W. Hypoxic pulmonary vasoconstriction: a multifactorial response? Am J Physiol Lung Cell Mol Physiol 2001;281:L314–7.
[36] Moudgil R, Michelakis ED, Archer SL. Hypoxic pulmonary vasoconstriction. J Appl Physiol 2005;98:390–403.

[37] Ward JP, Snetkov VA, Aaronson PI. Calcium, mitochondria and oxygen sensing in the pulmonary circulation. Cell Calcium 2004; 36:209–20.
[38] Robertson TP, Dipp M, Ward JP, Aaronson PI, Evans AM. Inhibition of sustained hypoxic vasoconstriction by Y-27632 in isolated intrapulmonary arteries and perfused lung of the rat. Br J Pharmacol 2000;131:5–9.
[39] Wang Z, Lanner MC, Jin N, Swartz D, Li L, Rhoades RA. Hypoxia inhibits myosin phosphatase in pulmonary arterial smooth muscle cells: role of Rho-kinase. Am J Respir Cell Mol Biol 2003;29:465–71.
[40] Wojciak-Stothard B, Tsang LY, Paleolog E, Hall SM, Haworth SG. Rac1 and RhoA as regulators of endothelial phenotype and barrier function in hypoxia-induced neonatal pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2006.
[41] Ward JP, Knock GA, Snetkov VA, Aaronson PI. Protein kinases in vascular smooth muscle tone-role in the pulmonary vasculature and hypoxic pulmonary vasoconstriction. Pharmacol Ther 2004;104: 207–31.
[42] Weissmann N, Ebert N, Ahrens M, Ghofrani HA, Schermuly RT, Hanze J, et al. Effects of mitochondrial inhibitors and uncouplers on hypoxic vasoconstriction in rabbit lungs. Am J Respir Cell Mol Biol 2003;29:721–32.
[43] Waypa GB, Chandel NS, Schumacker PT. Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ Res 2001;88:1259–66.
[44] Duchen MR. Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. J Physiol 1999;516(Pt 1):1–17.
[45] Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love–hate triangle. Am J Physiol Cell Physiol 2004;287:817–33.
[46] Han D, Antunes F, Canali R, Rettori D, Cadenas E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 2003;278:5557–63.
[47] Michelakis ED, Rebeyka I, Wu X, Nsair A, Thebaud B, Hashimoto K, et al. O_2 sensing in the human ductus arteriosus: regulation of voltage-gated K^+ channels in smooth muscle cells by a mitochondrial redox sensor. Circ Res 2002;91:478–86.
[48] Waypa GB, Schumacker PT. Hypoxic pulmonary vasoconstriction: redox events in oxygen sensing. J Appl Physiol 2005;98:404–14.
[49] Rounds S, McMurtry IF. Inhibitors of oxidative ATP production cause transient vasoconstriction and block subsequent pressor responses in rat lungs. Circ Res 1981;48:393–400.
[50] Archer SL, Huang J, Henry T, Peterson D, Weir EK. A redox-based O_2 sensor in rat pulmonary vasculature. Circ Res 1993;73:1100–12.
[51] Pitkanen S, Robinson BH. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. J Clin Invest 1996;98:345–51.
[52] Staniek K, Nohl H. Are mitochondria a permanent source of reactive oxygen species? Biochim Biophys Acta 2000;1460:268–75.
[53] Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. IUBMB Life 2001;52:159–64.
[54] Michelakis ED, Thebaud B, Weir EK, Archer SL. Hypoxic pulmonary vasoconstriction: redox regulation of O_2-sensitive K^+ channels by a mitochondrial O_2-sensor in resistance artery smooth muscle cells. J Mol Cell Cardiol 2004;37:1119–36.
[55] Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. J Biol Chem 2000; 275:25130–8.
[56] Leach RM, Hill HM, Snetkov VA, Robertson TP, Ward JP. Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor. J Physiol 2001;536:211–24.
[57] Weissmann N, Zeller S, Schafer RU, Turowski C, Ay M, Quanz K, et al. Impact of mitochondria and NADPH oxidases on acute and sustained hypoxic pulmonary vasoconstriction. Am J Respir Cell Mol Biol 2005.
[58] Paddenberg R, Ishaq B, Goldenberg A, Faulhammer P, Rose F, Weissmann N, et al. Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature. Am J Physiol Lung Cell Mol Physiol 2003;284:710–9.
[59] Chandel NS, Schumacker PT. Cells depleted of mitochondrial DNA (rho0) yield insight into physiological mechanisms. FEBS Lett 1999;454:173–6.
[60] Ferranti R, da Silva MM, Kowaltowski AJ. Mitochondrial ATP-sensitive K^+ channel opening decreases reactive oxygen species generation. FEBS Lett 2003;536:51–5.
[61] Barrientos A, Moraes CT. Titrating the effects of mitochondrial complex I impairment in the cell physiology. J Biol Chem 1999; 274:16188–97.
[62] Duchen MR, Biscoe TJ. Relative mitochondrial membrane potential and $[Ca^{2+}]_i$ in type I cells isolated from the rabbit carotid body. J Physiol 1992;450:33–61.
[63] Wang Q, Wang YX, Yu M, Kotlikoff MI. Ca(2+)-activated Cl^- currents are activated by metabolic inhibition in rat pulmonary artery smooth muscle cells. Am J Physiol 1997;273:520–30.
[64] Kang TM, Park MK, Uhm DY. Characterization of hypoxia-induced $[Ca^{2+}]_i$ rise in rabbit pulmonary arterial smooth muscle cells. Life Sci 2002;70:2321–33.
[65] Drummond RM, Tuft RA. Release of Ca^{2+} from the sarcoplasmic reticulum increases mitochondrial $[Ca^{2+}]$ in rat pulmonary artery smooth muscle cells. J Physiol 1999;516(Pt 1):139–47.
[66] Kang TM, Park MK, Uhm DY. Effects of hypoxia and mitochondrial inhibition on the capacitative calcium entry in rabbit pulmonary arterial smooth muscle cells. Life Sci 2003;72:1467–79.
[67] Hampl V, Herget J. Possible mechanisms of oxygen sensing in the pulmonary circulation. Physiol Res 1991;40:463–70.
[68] Yuan XJ, Tod ML, Rubin LJ, Blaustein MP. Hypoxic and metabolic regulation of voltage-gated K^+ channels in rat pulmonary artery smooth muscle cells. Exp Physiol 1995;80:803–13.
[69] Leach RM, Sheehan DW, Chacko VP, Sylvester JT. Energy state, pH, and vasomotor tone during hypoxia in precontracted pulmonary and femoral arteries. Am J Physiol Lung Cell Mol Physiol 2000; 278:294–304.
[70] Buescher PC, Pearse DB, Pillai RP, Litt MC, Mitchell MC, Sylvester JT. Energy state and vasomotor tone in hypoxic pig lungs. J Appl Physiol 1991;70:1874–81.
[71] Fisher AB, Dodia C. Lung as a model for evaluation of critical intracellular PO_2 and PCO. Am J Physiol 1981;241:E47–50.
[72] Bell EL, Emerling BM, Chandel NS. Mitochondrial regulation of oxygen sensing. Mitochondrion 2005;5:322–32.
[73] Wiener CM, Sylvester JT. Effects of glucose on hypoxic vasoconstriction in isolated ferret lungs. J Appl Physiol 1991;70:439–46.
[74] Wiener CM, Sylvester JT. Effects of insulin, glucose analogues, and pyruvate on vascular responses to anoxia in isolated ferret lungs. J Appl Physiol 1993;74:2426–31.
[75] Evans AM, Mustard KJ, Wyatt CN, Peers C, Dipp M, Kumar P, et al. Does AMP-activated protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O_2-sensing cells? J Biol Chem 2005;280:41504–11.
[76] Wilson HL, Dipp M, Thomas JM, Lad C, Galione A, Evans AM. ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase act as a redox sensor. A primary role for cyclic ADP-ribose in hypoxic pulmonary vasoconstriction. J Biol Chem 2001;276:11180–8.
[77] Acker H. The oxygen sensing signal cascade under the influence of reactive oxygen species. Philos Trans R Soc Lond B Biol Sci 2005;360:2201–10.
[78] Streller T, Huckstorf C, Pfeiffer C, Acker H. Unusual cytochrome *a*592 with low PO_2 affinity correlates as putative oxygen sensor with rat carotid body chemoreceptor discharge. FASEB J 2002;16:1277–9.

[79] Okabe E, Tsujimoto Y, Kobayashi Y. Calmodulin and cyclic ADP-ribose interaction in Ca^{2+} signaling related to cardiac sarcoplasmic reticulum: superoxide anion radical-triggered Ca^{2+} release. Antioxid Redox Signal 2000;2:47–54.
[80] Bokoch GM, Knaus UG. NADPH oxidases: not just for leukocytes anymore! Trends Biochem Sci 2003;28:502–8.
[81] Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol 2003;285:277–97.
[82] Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494–501.
[83] Banfi B, Clark RA, Steger K, Krause KH. Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J Biol Chem 2003;278:3510–3.
[84] Geiszt M, Lekstrom K, Witta J, Leto TL. Proteins homologous to p47phox and p67phox support superoxide production by NAD(P)H oxidase 1 in colon epithelial cells. J Biol Chem 2003;278:20006–12.
[85] Shmelzer Z, Haddad N, Admon E, Pessach I, Leto TL, Eitan-Hazan Z, et al. Unique targeting of cytosolic phospholipase A2 to plasma membranes mediated by the NADPH oxidase in phagocytes. J Cell Biol 2003;162:683–92.
[86] Weissmann N, Voswinckel R, Hardebusch T, Rosseau S, Ghofrani HA, Schermuly R, et al. Evidence for a role of protein kinase C in hypoxic pulmonary vasoconstriction. Am J Physiol 1999;276:90–5.
[87] Matsuzaki I, Chatterjee S, Debolt K, Manevich Y, Zhang Q, Fisher AB. Membrane depolarization and NADPH oxidase activation in aortic endothelium during ischemia reflect altered mechanotransduction. Am J Physiol Heart Circ Physiol 2005;288:336–43.
[88] Henderson LM, Chappell JB, Jones OT. The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an H+ channel. Biochem J 1987;246:325–9.
[89] Fu XW, Wang D, Nurse CA, Dinauer MC, Cutz E. NADPH oxidase is an O2 sensor in airway chemoreceptors: evidence from K^+ current modulation in wild-type and oxidase-deficient mice. Proc Natl Acad Sci U S A 2000;97:4374–9.
[90] Thomas HM III, Carson RC, Fried ED, Novitch RS. Inhibition of hypoxic pulmonary vasoconstriction by diphenyleneiodonium. Biochem Pharmacol 1991;42:9–12.
[91] Grimminger F, Weissmann N, Spriestersbach R, Becker E, Rosseau S, Seeger W. Effects of NADPH oxidase inhibitors on hypoxic vasoconstriction in buffer-perfused rabbit lungs. Am J Physiol 1995; 268:747–52.
[92] Marshall C, Mamary AJ, Verhoeven AJ, Marshall BE. Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. Am J Respir Cell Mol Biol 1996;15:633–44.
[93] Wolin MS, Burke-Wolin TM, Mohazzab H. Roles for NAD(P)H oxidases and reactive oxygen species in vascular oxygen sensing mechanisms. Respir Physiol 1999;115:229–38.
[94] Weissmann N, Grimminger F, Voswinckel R, Conzen J, Seeger W. Nitroblue tetrazolium inhibits but does not mimic hypoxic vasoconstriction in isolated rabbit lungs. Am J Physiol 1998;274:721–7.
[95] Majander A, Finel M, Wikstrom M. Diphenyleneiodonium inhibits reduction of iron–sulfur clusters in the mitochondrial NADH-ubiquinone oxidoreductase (Complex I). J Biol Chem 1994;269: 21037–42.
[96] Weir EK, Reeve HL, Cornfield DN, Tristani-Firouzi M, Peterson DA, Archer SL. Diversity of response in vascular smooth muscle cells to changes in oxygen tension. Kidney Int 1997;51:462–6.
[97] Weissmann N, Tadic A, Hanze J, Rose F, Winterhalder S, Nollen M, et al. Hypoxic vasoconstriction in intact lungs: a role for NADPH oxidase-derived H(2)O(2)? Am J Physiol Lung Cell Mol Physiol 2000;279:683–90.
[98] Weissmann N, Schermuly RT, Ghofrani HA, Haenze J, Goyal P, Kuzkaya N, et al. Hypoxic pulmonary vasoconstriction-triggered by an increase in reactive oxygen species? Novartis Found Symp 2005;272:196–213.
[99] Ichinose F, Ullrich R, Sapirstein A, Jones RC, Bonventre JV, Serhan CN, et al. Cytosolic phospholipase A(2) in hypoxic pulmonary vasoconstriction. J Clin Invest 2002;109:1493–500.
[100] Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, et al. O_2 sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. Proc Natl Acad Sci U S A 1999;96:7944–9.
[101] Wolin MS, Ahmad M, Gupte SA. Oxidant and redox signaling in vascular oxygen sensing mechanisms: basic concepts, current controversies, and potential importance of cytosolic NADPH. Am J Physiol Lung Cell Mol Physiol 2005;289:159–73.
[102] Grimminger F, Spriestersbach R, Weissmann N, Walmrath D, Seeger W. Nitric oxide generation and hypoxic vasoconstriction in buffer-perfused rabbit lungs. J Appl Physiol 1995;78:1509–15.
[103] Spriestersbach R, Grimminger F, Weissmann N, Walmrath D, Seeger W. On-line measurement of nitric oxide generation in buffer-perfused rabbit lungs. J Appl Physiol 1995;78:1502–8.
[104] Weissmann N, Winterhalder S, Nollen M, Voswinckel R, Quanz K, Ghofrani HA, et al. NO and reactive oxygen species are involved in biphasic hypoxic vasoconstriction of isolated rabbit lungs. Am J Physiol Lung Cell Mol Physiol 2001;280:638–45.
[105] Gupte SA, Kaminski PM, Floyd B, Agarwal R, Ali N, Ahmad M, et al. Cytosolic NADPH may regulate differences in basal Nox oxidase-derived superoxide generation in bovine coronary and pulmonary arteries. Am J Physiol Heart Circ Physiol 2005;288:13–21.
[106] Gupte SA, Okada T, McMurtry IF, Oka M. Role of pentose phosphate pathway-derived NADPH in hypoxic pulmonary vasoconstriction. Pulm Pharmacol Ther 2006;19:303–9.
[107] Weissmann N, Kuzkaya N, Fuchs B, Tiyerili V, Schafer RU, Schutte H, et al. Detection of reactive oxygen species in isolated, perfused lungs by electron spin resonance spectroscopy. Respir Res 2005;6:86.
[108] Sylvester JT. Hypoxic pulmonary vasoconstriction: a radical view. Circ Res 2001;88:1228–30.
[109] Sham JS. Hypoxic pulmonary vasoconstriction: ups and downs of reactive oxygen species. Circ Res 2002;91:649–51.
[110] Archer SL, Nelson DP, Weir EK. Simultaneous measurement of O_2 radicals and pulmonary vascular reactivity in rat lung. J Appl Physiol 1989;67:1903–11.
[111] Weissmann N, Vogels H, Schermuly RT, Ghofrani HA, Hanze J, Fink L, et al. Measurement of exhaled hydrogen peroxide from rabbit lungs. Biol Chem 2004;385:259–64.
[112] Killilea DW, Hester R, Balczon R, Babal P, Gillespie MN. Free radical production in hypoxic pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2000;279:408–12.
[113] Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, et al. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 2005;1:401–8.
[114] Weissmann N, Seeger W, Conzen J, Kiss L, Grimminger F. Effects of arachidonic acid metabolism on hypoxic vasoconstriction in rabbit lungs. Eur J Pharmacol 1998;356:231–7.
[115] Zhu D, Birks EK, Dawson CA, Patel M, Falck JR, Presberg K, et al. Hypoxic pulmonary vasoconstriction is modified by P-450 metabolites. Am J Physiol Heart Circ Physiol 2000;279:1526–33.
[116] Yuan XJ, Tod ML, Rubin LJ, Blaustein MP. Inhibition of cytochrome *P*-450 reduces voltage-gated K^+ currents in pulmonary arterial myocytes. Am J Physiol 1995;268:259–70.
[117] Jacobs ER, Zeldin DC. The lung HETEs (and EETs) up. Am J Physiol Heart Circ Physiol 2001;280:H1–10.
[118] Hoshi T, Lahiri S. Cell biology. Oxygen sensing: it's a gas!. Science 2004;306:2050–1.
[119] Williams SE, Wootton P, Mason HS, Bould J, Iles DE, Riccardi D, et al. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. Science 2004;306:2093–7.
[120] Kemp PJ. Hemeoxygenase-2 as an O_2 sensor in K^+ channel-dependent chemotransduction. Biochem Biophys Res Commun 2005;338:648–52.
[121] Patel AJ, Honore E. Molecular physiology of oxygen-sensitive potassium channels. Eur Respir J 2001;18:221–7.