Vascular adaptations to hypoxia: molecular and cellular mechanisms regulating vascular tone

Michael L. Paffett and Benjimen R. Walker

Abstract

Several molecular and cellular adaptive mechanisms to hypoxia exist within the vasculature. Many of these processes involve oxygen sensing which is transduced into mediators of vasoconstriction in the pulmonary circulation and vasodilation in the systemic circulation. A variety of oxygen-responsive pathways, such as HIF (hypoxia-inducible factor)-1 and HOs (haem oxygenases), contribute to the overall adaptive process during hypoxia and are currently an area of intense research. Generation of ROS (reactive oxygen species) may also differentially regulate vascular tone in these circulations. Potential candidates underlying the divergent responses between the systemic and pulmonary circulations may include Nox (NADPH oxidase)-derived ROS and mitochondrial-derived ROS. In addition to alterations in ROS production governing vascular tone in the hypoxic setting, other vascular adaptations are likely to be involved. HPV (hypoxic pulmonary vasoconstriction) and CH (chronic hypoxia)-induced alterations in cellular proliferation, ionic conductances and changes in the contractile apparatus sensitivity to calcium, all occur as adaptive processes within the vasculature.

1To whom correspondence should be addressed (email bwalker@salud.unm.edu).
Introduction

The response to decreased $PO_2$ (partial pressure of oxygen) or hypoxia within the vasculature is complex and associated with many acute and chronic adaptations. In addition, the pulmonary and systemic circulations respond very differently to hypoxia although they may share common cellular signalling events initiated by low $PO_2$. Arteries from both circulations contain VSM (vascular smooth muscle) that dictates the active tone of the vessels and a metabolically active endothelium. Due to its proximity to the endothelium, the VSM receives paracrine cues in response to decreasing $PO_2$, as well as sensing hypoxia directly through various cellular mechanisms. The endothelium not only serves as a protective barrier from the circulating blood, but also is a rich source of vasoactive substances that determine vascular tone under a variety of conditions, including hypoxia. A variety of molecular pathways and cellular sensors respond to hypoxia which activate homeostatic mechanisms in the cardiovascular and respiratory systems. These oxygen-sensing capabilities within the vasculature often bring about change in gene expression and signal transduction pathways that uniquely determine the haemodynamic state within a given vascular bed that can be deemed adaptive in some circumstances and/or pathological in others depending upon the magnitude and duration of the hypoxic stimulus.

Vascular oxygen-sensing mechanisms

Hypoxia-inducible gene expression

One of the most studied transcription factors responsive to reduced $PO_2$ is HIF (hypoxia-inducible factor)-1. Analysis of purified HIF-1 revealed a heterodimeric protein containing HIF-1α and HIF-1β subunits also referred to as ARNT (aryl hydrocarbon receptor nuclear translocator). Consequently, protein structure analysis has demonstrated that each protein in this heterodimer contains a bHLH (basic helix-loop-helix) domain capable of binding to the promoter region and activating transcription of a variety of genes under hypoxic conditions [1]. However, under normoxic conditions the VHL (von Hippel-Lindau) tumour suppressor protein binds to two hydroxy-proline residues at Pro402 and Pro564 located in the transactivation domain of HIF-1α and this complex is then recognized by the E3 ubiquitin ligase which routes this complex through the 26S proteasomal degradation pathway (left-hand panel of Figure 1). Furthermore, a third hydroxylation site at Asp803 serves to inhibit CBP [CREB (cAMP-response-element-binding protein)-binding protein] and p300 binding, therefore restricting the activity of these transcriptional co-activators [2]. Hydroxylation of these residues is carried out by prolyl and aaparaginyl hydroxylases in which molecular oxygen serves as the rate-limiting substrate for this reaction. Under hypoxic conditions hydroxylase activity subsides and the loss of hydroxylation at these residues results in a stable HIF-1α, which is then capable of recruiting p300/CBP, binding to the promoter region
containing a HRE (hypoxia-response element) and inducing gene expression (right-hand panel of Figure 1). For example, numerous studies have shown HIF-1 dependent expression of ET-1 (endothelin-1) in the vascular endothelium resulting in elevated plasma ET-1 during hypoxic conditions [3]. In turn, ET-1 has pleiotropic effects in which it can cause vasoconstriction, alterations in VSM calcium sensitivity and proliferation of pulmonary VSM leading to increased vascular resistance. These alterations in vascular function associated with CH (chronic hypoxia) are discussed in more detail below. Table 1 lists a number of HIF-responsive genes and the proposed roles for the encoded protein within the vasculature. Furthermore, regulation of HIF-1α by agonist-induced ROS (reactive oxygen species) production may occur in both pulmonary and aortic VSM cells under non-hypoxic conditions, since pretreatment with antioxidants averts HIF-1α accumulation and expression of HIF-1-dependent genes [4,5]. These findings clearly suggest a role for ROS in regulating HIF-1α activity and implicate ROS as a modulator of HIF-1 pathways within pulmonary and systemic arteries. Although these studies did not investigate the effects of hypoxia on ROS-dependent HIF-1 stabilization, these observations do provide insight into HIF-1α regulation by ROS production.

Figure 1. Mechanism of oxygen-dependent degradation of HIF-1
Oxygen-dependent prolyl hydroxylation allows the VHL and E3 ligase complex to associate with HIF-1 in order for the E3 ligase to ubiquitinate the HIF-1α subunit. Asparaginyl hydroxylation of HIF-1 prevents binding of the transcription factor p300/CBP. During hypoxic conditions, prolyl and asparaginyl hydroxylase activity is reduced, thus facilitating the translocation of this complex to the nucleus where HIF-1 then serves as a transcription factor. OH, hydroxylation sites for oxygen-dependent prolyl and asparaginyl hydroxylases; Ub, ubiquitin.
Interestingly, ROS production is also altered in the hypoxic setting [6]. The effects of hypoxia on ROS production and the accompanying divergent vaso-motor responses between vascular beds will be discussed in the next section.

**Nox (NADPH oxidase)**

A rapidly emerging body of evidence suggests that Nox may be a putative oxygen sensor within the vasculature. Noxs consist of a membrane-bound phox (phagocytic oxidase), cytosolic regulatory subunits and a small GTPase. The flavin- and haem-binding glycoprotein (gp91phox or Nox2) and p22phox subunits constitute the membrane-associated flavocytochrome b558 initially discovered by spectral analysis. Binding of the regulatory cytosolic p40phox, p47phox and p67phox proteins increase the activity of Nox by enhancing flux through the core oxidase (Figure 2). The small GTP-binding protein Rac1/2 also imposes a regulatory function on Nox activity. Several Nox isoforms have been identified, of which Nox1–4 lack intracellular calcium-binding sites; however Nox5

<table>
<thead>
<tr>
<th>Protein</th>
<th>Role(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic receptor (β₁)</td>
<td>Apoptosis and cardiovascular development</td>
</tr>
<tr>
<td>ET-1</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td></td>
<td>Vascular remodelling</td>
</tr>
<tr>
<td></td>
<td>Altered VSM calcium sensitivity</td>
</tr>
<tr>
<td>HO-1</td>
<td>Vasodilation and ROS scavenging</td>
</tr>
<tr>
<td>Inducible NO synthase</td>
<td>Vasodilation and inflammation</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>Angiogenesis and endothelial permeability</td>
</tr>
</tbody>
</table>

Table 1. Partial list of HIF-1α-responsive genes and associated roles within the vasculature

![Figure 2. Nox and ancillary proteins that modulate electron flux generating superoxide anion](attachment:figure2.png)

The core oxidase composition is composed of gp91phox and p22phox subunits located at the plasma membrane. GTP-bound Rac, p47phox, p40phox and p67phox have been identified as modulators of Nox activity. NADPH(H⁺) and molecular oxygen serve as substrates for Nox-generating superoxide anion and NADP⁺ and H₂O. The two-step electron transfer generating 2 moles of superoxide anion from NADPH(H⁺) and FAD to convert haemox to hemred in the gp91phox subunit is indicated in the inset.

© The Authors Journal compilation © 2007 Biochemical Society
has four calcium-binding EF hands near the N-terminus. The EF-hand motifs impart a calcium sensitivity to the enzyme and increases in cytosolic calcium augment ROS production [7]. Nox homologues of the first identified gp91phox subunit possess a wide range of sequence similarities and are found in a variety of cell types between species and cultured cell lines. Additionally, the novel isoform of p47phox termed Nox1 (Nox organizer 1) has unique structural characteristics which include a deletion of an autoinhibitory domain and differs in its lipid-binding specificity compared with p47phox [8]. Additionally, the p67phox homologue, p51phox or Noxa1 (NADPH oxidase activator 1) has a high degree of sequence homology to its parent isoform and regulates Nox activity by interacting with the flavocytochrome complex [9]. The existence of these two ancillary protein variants gives rise to uniquely composed Noxs which may offer further insight regarding Nox function. Interestingly, gp91phox and the cytosolic regulators (p40phox, p47phox and p67phox) exist in the VSM [10,11] and participate in ROS production following receptor stimulation. Nox requires molecular oxygen and NADPH as substrates which results in the generation of the superoxide anion (O₂⁻) which is then capable of permeating the plasma membrane via anionic channels and being further reduced to H₂O₂ by SOD (superoxide dismutase). Either of these ROS can modify a variety of signal transduction pathways that regulate vascular tone and expression of specific genes known to affect vascular function. Hypoxic transitions were initially predicted to limit oxidant (i.e. O₂⁻ and H₂O₂) production and promote a more reduced state within the cytosol. Although this appears to be the case in systemic arteries which generally dilate in response to hypoxia, pulmonary arteries constrict and Nox activity appears to increase in this setting.

**HO (haemooxygenase)**

HOs represent another postulated cellular oxygen sensor within many different cell types, including the vasculature. HOs catalyse the oxidation of haem to equimolar parts of carbon monoxide, Bv (biliverdin) and free ferrous iron (Figure 3). Three known isoforms, HO-1, HO-2 and HO-3 have been identified to date. HO-1 is largely considered to be the only inducible isoform. HO-1 expression is associated with elevated oxidative stress, shear stress, as well as with hypoxia. HO-2 is constitutively expressed in neurons and the vascular endothelium, with only a limited number of physiological activators, including adrenal glucocorticoids and protein kinase C. The most recently identified isoform, HO-3, appears to be incapable of haem degradation and is thought to primarily serve as a haem-sensing and/or-binding protein [12]. Since HOs require oxygen to degrade haem, they are theorized to be putative oxygen sensors. The majority of attention within the field of vascular physiology centres on the vasodilatory role of the HO product, carbon monoxide, on vascular tone, specifically within the VSM. Carbon monoxide acts upon the haem-containing protein sGC (soluble guanylate cyclase) to stimulate cyclic GMP production [13] leading to protein kinase G-dependent vasodilation in systemic arterial beds [14]. Furthermore, the
potent vasodilator NO (nitric oxide), produced by the vascular endothelium also causes vasodilation by stimulating sGC activity in a similar fashion. Interestingly, increases in HO-1 expression occur following Nox inhibition, presumably by signalling via protein kinase B and p38 MAPK (mitogen-activated protein kinase) signalling pathways [15]. These results suggest that HO-1 expression is sensitive to a decrease in Nox-associated ROS formation and are consistent with similar findings where HO-1 expression increased after treatment with the anti-oxidant pyrrolidine dithiocarbamate [16]. Although these findings suggest up-regulation of HO-1 gene expression in monocytes by scavenging endogenous ROS, rat aortic VSM cells show a ROS-dependent increase in HO-1 expression through activation of p42/44 MAPK pathways and AP-1 (activator protein-1) [17]. These discrepancies in HO-1 gene expression may be due to tissue-specific differences in redox-sensitive transcriptional pathways or non-specific effects of the ROS-scavenging compounds utilized in previous studies. In addition to the importance of redox state and HO-1 transcriptional activity, the conversion of \( \text{Bv} \) to the potent anti-oxidant \( \text{Br} \) (bilirubin) by \( \text{BvR} \) (Bv reductase) is hypothesized to be an efficient ROS scavenging system [18]. The accumulation of \( \text{Br} \) provides an anti-oxidant pool to scavenge endogenous ROS. Several studies demonstrate the protective nature of HO-1 induction via pharmacological or gene transfer techniques in which blood pressure restoration and endothelial oxidative injury were curtailed in

**Figure 3. Role of HO in oxygen-sensing and vascular control**

HOs utilize haem and molecular oxygen to generate carbon monoxide, \( \text{Br} \) and Fe(II). Carbon monoxide can then activate sGC to elicit protein kinase G-dependent relaxation. \( \text{Bv} \) is reduced to the potent anti-oxidant \( \text{Br} \) by \( \text{BvR} \). Fe(II) sequestration occurs by binding to ferritin.

\[
\begin{align*}
\text{HO} & : 3 \text{O}_2 + \text{Haem} \rightarrow 3 \text{NADPH} (H^+) + 3 \text{NADP}^+ + 3 \text{H}_2\text{O} + \text{CO} + \text{Fe(II)} \\
\text{Bv} & : \text{BvR} + \text{Fe(II)} \rightarrow \text{Bv} + \text{BvR} + \text{Fe(II)} \\
\text{ROS} & : \text{Bv} + \text{NADPH} (H^+) \rightarrow \text{BvR} + \text{NADP}^+ \\
\text{sGC} & : \text{cGMP} \\
\text{PKG} & : \text{cGMP}
\end{align*}
\]
renal models of hypertension and hyperglycaemic rats [19]. An interesting aspect of haem degradation is that under conditions that induce HO-1, such as hypoxia, this putative oxygen sensor may provide protective antioxidant effects against increased ROS formation associated with various acute and chronic disease states.

Mitochondria as oxygen sensors

Cellular respiration requires that molecular oxygen serves as a terminal electron sink in the distal electron transport chain to establish a proton-motive force necessary for ATP production. Similar to Nox, mitochondria generate ROS as metabolic by-products. As NADH and FADH are generated from the tricarboxylic acid cycle, electrons are transferred through iron-sulfur centres located in the electron transport chain. During this process of oxidation and reduction, O$_2^-$ is produced at several points along the electron transport chain. ROS production occurs at complex I and at two distinct redox sites of the quinone cycle at complex III. Although measurement of basal mitochondrial ROS production is difficult, from mitochondria in pulmonary arteries conflicting evidence exists regarding the effect of hypoxia on this variable. Various levels of mitochondrial SODs have also been investigated in the systemic and pulmonary arterial circulations and are proposed to influence the level of ROS production in these vascular beds [20]. It has also been postulated that during hypoxia a build-up of NADH ensues due to the reduction in the terminal electron acceptor, molecular oxygen [21]. These clues may be useful in determining the overall mechanisms responsible for the different responses to hypoxia in these vascular beds.

Hypoxia: ROS and redox state

The role of ROS during hypoxia

Although it has been a long-held belief that ROS are detrimental to cellular physiology, more recent evidence suggests that ROS are critical mediators in many signalling pathways. As an adaptive process to hypoxia, reduced PO$_2$ must be sensed and then transduced by an effector molecule that brings about change within a system. It is typically thought that systemic arteries dilate and pulmonary arteries constrict in response to hypoxia. Given the likelihood of similar oxygen-sensing capabilities between tissues, unique downstream effectors may underlie these divergent responses. This may be due to fundamental differences in ROS production between these circulations under normoxic conditions (Table 2). Interestingly, basal production of NADPH is greater in bovine pulmonary arteries than in coronary arteries presumably due to greater expression of G6PD (glucose-6-phosphate dehydrogenase) [22]. In contrast to the pulmonary circulation, it is hypothesized that normoxic NADPH production and G6PD expression are diminished in systemic arteries by competition between glycolytic and pentose phosphate pathways [23]. Concomitant decreases in O$_2^-$ and H$_2$O$_2$
production promoting relaxation are also observed in this systemic vascular bed [20]. Furthermore, these investigators hypothesize that the decrease in basal NADPH production may in turn be responsible for the accompanying vasodilation observed in systemic vascular beds during hypoxia. This diminished availability of substrate (NADPH) for Nox may be a critical variable that distinguishes coronary artery vasodilation from pulmonary artery vasoconstriction during hypoxia. The differences in basal ROS production between these circulations may provide insight into the contrasting hypoxic vasomotor responses commonly observed.

A general assumption in redox chemistry is that when \( P_O2 \) decreases, a subsequent reduction in free-radical generation follows by virtue of the fact that there is less substrate to generate ROS. However, this prediction is not observed in pulmonary VSM cells in which increased NADPH-dependent superoxide formation is observed [24] during hypoxia. Furthermore, recent evidence suggests that CH may actually lead to increased \( O_2^- \) production, presumably by increased activity of NADPH oxidase [25]. These investigators observed enhanced agonist-induced vasoconstriction and increased muscularization of intrapulmonary arteries following CH in gp91\(^{phox} \) wild-type mice compared with the gp91\(^{phox} \) knockout independent of any changes in Nox-subunit mRNA expression. The divergent vasomotor responses to hypoxia between the pulmonary and coronary (systemic) circulations appear to be associated with fundamentally unique metabolic and signalling pathways that account for differential regulation of vascular resistance in response to hypoxia between these beds.

In addition to various responses of Nox during hypoxia, conflicting effects of hypoxia on mitochondrial ROS generation in pulmonary arteries may exist. For example, hypoxia has been shown to augment ROS production and cause vasoconstriction that is attenuated by the distal complex III inhibitor myxothiazol [26]. In addition to redox inhibition of the mitochondrial electron transport chain, others have demonstrated the loss of contractile response to hypoxia following the depletion of essential electron transport chain subunits with a two week exposure to ethidium bromide [27]. It has been further hypothesized that a decrease in hypoxia-induced ROS production leads to a change in the redox equilibrium toward a more reduced state. In contrast, others have observed a decreased ROS production during hypoxia or following complex I and proximal complex III inhibition [28,29]. Therefore conflicting

| Table 2. Divergent effects of hypoxia on ROS production in the pulmonary and systemic circulations |
|---|---|---|---|
| Arterial bed | ROS source | Mitochondrial | Extra-mitochondrial |
| Pulmonary | Increase/decrease | Increase | Increase Vasoconstriction |
| Systemic | Increase | Decrease | Decrease Vasodilation |

© The Authors Journal compilation © 2007 Biochemical Society
data exist regarding the influence of hypoxia on ROS production and the role of these mitochondrial and extra-mitochondrial effector pathways in the regulation of vascular tone.

**Effect of redox state on VSM potassium conductances**

As discussed above, hypoxia appears to differentially impact the production of ROS and perturb the equilibrium of cellular reducing equivalents such as NADPH. The primary agent for maintaining redox homoeostasis is glutathione. This compound can exist either in the reduced (GSH) or oxidized state (GSSG). The reduced form of glutathione with glutathione peroxidase are critical components that maintain appropriate levels of endogenous peroxides. As part of the regenerative cycle to replenish GSH from GSSG, glutathione reductase activity is largely determined from cytosolic NADPH concentrations. The associated changes in cellular metabolism associated with the cytosolic NADPH(H+)/NADP+ ratio and ROS production during hypoxia are important variables that can alter redox homoeostasis and ultimately lead to functional changes within the vasculature. For example, the Kv (voltage-sensitive potassium) channels Kv1.5 and Kv2.1 have been implicated in HPV (hypoxic pulmonary vasoconstriction) [30]. It is hypothesized that hypoxia increases the reduced state within the VSM resulting in a decreased open probability of these channels leading to depolarization of the sarcolemma and activation of L-type VGCCs (voltage-gated calcium channels). The increase in open probability of VGCCs results in an increased global VSM calcium concentration and constriction of the artery. Although this potential effect of hypoxia on ROS production and an overall increase in the cellular reducing state in pulmonary arteries results in vasoconstriction, renal arteries show increased ROS production and exhibit vasodilation suggesting increased outward potassium conductances in this setting [20]. Furthermore, potassium channel conductances in the ductus arteriosus are thought to decrease in the face of increasing PO2 [31]. These examples further demonstrate the differential effects of hypoxia and reducing state in pulmonary and systemic arterial bed. The proposed role of altered cellular redox state to differentially influence potassium channels and thus vasomotor responses between the pulmonary and systemic arterial circulations is intriguing, however this picture is still not complete.

**Effects of acute hypoxia in pulmonary arteries**

**HPV**

The pulmonary circulation is typically characterized by its low resistance, low pressure profile that is advantageous for gas exchange between the alveoli and surrounding capillary beds. Even under normal conditions there is a small proportion of respiratory airways that are not adequately ventilated and therefore local regions of hypoxia develop. The response to this localized decrease in PO2 is pulmonary arterial vasoconstriction that diverts blood flow to better
ventilated areas, thereby matching ventilation and perfusion. However, global hypoxia observed in many pulmonary disease states and presence at high altitude leads to generalized pulmonary artery vasoconstriction and the eventual development of pulmonary hypertension. This response is unique to the pulmonary arterial circulation and is conserved in the respiratory organs of a variety of phylogenetically unrelated species, demonstrating its physiological importance in regulating gas exchange. Many hypotheses and potential mechanisms have been proposed to explain this phenomenon such as redox inhibition of Kv channels leading to VGCC-dependent calcium influx and contraction (described above). However, other potential mechanisms for HPV may also contribute to the response. For example, hypoxia-induced calcium release from the sarcoplasmic reticulum of VSM cells has been linked to increased cyclic ADP ribose levels in small pulmonary arteries [32]. It is proposed that cyclic ADP ribose stimulates the release of calcium by ryanodine receptors. Cyclic ADP ribose stability appears to be regulated by cytosolic NADH levels and levels of this reduced compound are elevated during acute hypoxia. Others have shown a link between lowering of VSM intracellular calcium stores and subsequent activation of store-operated calcium entry in intrapulmonary arteries [33]. Furthermore, removal of the endothelium reduces HPV by inhibition of Rho kinase sensitization of the VSM contractile apparatus to calcium [34]. These results suggest that hypoxia elicits the release of an endothelium-derived substance that modulates the sensitivity of the contractile apparatus to calcium in the underlying VSM. ET-1 may be the endothelium-derived substance responsible for this effect since administration of exogenous ET-1 restores HPV following endothelial disruption [35]. It is clear from the above discussion and numerous other reports in the literature, that HPV may result from the interaction of several signalling pathways.

**Effects of CH in pulmonary arteries**

Conditions associated with CH, such as COPD (chronic obstructive pulmonary disease) or prolonged residence at high altitude, result in several vascular adaptations within the arterial segment of the lung that contribute to the development of pulmonary hypertension. These changes include vascular remodelling of the arterial segment as well as enhanced vasoconstrictor reactivity, both of which increase pulmonary vascular resistance leading to hypertension.

**Structural alterations: remodelling of the VSM**

Medial and intimal remodelling in response to CH increases pulmonary vascular resistance by reducing the luminal diameter of the vessel. This encroachment is characterized by increased growth and proliferation of the VSM. As previously discussed, a key factor in CH-induced arterial VSM remodelling appears to centre on HIF-dependent transcriptional up-regulation of ET-1. ET-1 has many effects on the underlying VSM. For example, upon binding to VSM ET_{A} receptors, ET-1 causes a rise in VSM intracellular calcium and
subsequent vasoconstriction. In addition to evoking acute vasoconstriction, elevated VSM calcium also activates the calcium-sensitive transcription factor CBP resulting in up-regulation of growth specific genes [36]. This in turn leads to increased wall thickening and muscularization of previously thin-walled arteries in the lung [37]. These proliferative responses are also seen in the intimal layer (endothelium) in patients with mild COPD [38]. This proliferative response of the endothelium has been correlated to a higher degree of endothelial dysfunction [39], thereby leading to a more hypertensive state within the lungs of COPD patients. In addition to the hypertrophic response of the VSM and endothelium, increased deposition of the ECM (extracellular matrix) proteins collagen and elastin may result through greater expression of TGF-β (transforming growth factor-β). The role of TGF-β has been examined where a dominant-negative mutation in the TGF-β receptor impairs the development of CH-induced pulmonary hypertension, presumably by limiting arterial remodelling [40]. The phenotypic composition of the surrounding adventitia is also altered [41] in which the proportion of fibroblast sub-populations shifts to a myofibroblast dominating phenotype. Thus it is apparent that hyperplasia and hypertrophy of the cellular elements of the vascular wall leads to structural alterations that influence the degree of pulmonary hypertension in the setting of CH and/or COPD.

VSM depolarization and decreased Kv channel expression

$E_m$ (resting membrane potential) is an efficient regulator of calcium influx as discussed above in the case of the redox effects on Kv channel activity during HPV. Interestingly, VSM from small pulmonary arteries exposed to CH as well as VSM exposed to CH in culture have a more depolarized $E_m$ than controls [42,43], thus leading to increased calcium influx and vasoconstriction. It appears that this depolarization is due to down-regulation of several isoforms of Kv channels in the VSM [44]. Furthermore, inhibition of potassium channel activity and/or expression may also inhibit apoptosis [45], thus potentially contributing to the more growth-responsive state observed in remodelling of the VSM during CH. Therefore decreased potassium channel expression during CH appears to alter the VSM $E_m$ but also enhances vascular remodelling in this setting.

Alterations in calcium sensitivity of the contractile apparatus

The small GTPase RhoA and its downstream effector Rho kinase have gained considerable attention with regard to the pathogenesis of CH-induced pulmonary hypertension. The RhoA/Rho kinase pathway plays an important role in regulating the contractile state of VSM by affecting the degree of MLC (myosin light chain) phosphorylation through inhibition of MLC phosphatase activity. Regulation of cross-bridge cycling is governed by a balance between activities of the calcium/calmodulin-dependent MLC kinase and calcium-independent MLC phosphatase. Activation of the RhoA/Rho kinase pathway
through ligand-dependent and ligand-independent mechanisms plays a significant role in the sustained vasoconstriction following CH. For example, Rho kinase inhibition, but not L-type VGCC inhibition elicits vasodilation in lungs from CH rats, suggesting increased VSM calcium sensitivity contributes to pulmonary hypertension in this setting [46,47].

Conclusion

Although the oxygen-sensing mechanisms outlined above exist in both the pulmonary and systemic vasculatures, the specific means by which they differentially affect vascular tone is still unclear. The role of these putative mechanisms in hypoxia-induced systemic vasodilation and pulmonary vasoconstriction continues to be an area of intense research and debate. Advances in our understanding of vascular adaptations to hypoxia will most likely centre on the role of redox state in oxygen-sensing within the vasculature, and how ROS influences signalling pathways that determine vascular tone.

Summary

• A variety of oxygen-sensing mechanisms exist that alter the reactivity of the vasculature.
• ROS production from various sites within the mitochondrial electron transport chain as well as extra-mitochondrial sources appear to play unique roles in pulmonary and systemic arteries.
• The redox state of the vessel wall may vary between these two circulations and these differences may provide further insight into how each circulation responds and adapts to hypoxia.
• A variety of structural and electrophysiological alterations take place following CH in the pulmonary vasculature that contributes to the development of pulmonary hypertension.

References


