55th Bowditch Lecture: Effects of chronic hypoxia on the pulmonary circulation: Role of HIF-1

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Shimoda L.A. 55th Bowditch Lecture: Effects of chronic hypoxia on the pulmonary circulation: role of HIF-1. J Appl Physiol 113: 1343–1352, 2012. First published August 23, 2012; doi:10.1152/japplphysiol.00843.2012.—When exposed to chronic hypoxia (CH), the pulmonary circulation responds with enhanced contraction and vascular remodeling, resulting in elevated pulmonary arterial pressures. Our work has identified CH-induced alterations in the expression and activity of several ion channels and transporters in pulmonary vascular smooth muscle that contribute to the development of hypoxic pulmonary hypertension and uncovered a critical role for the transcription factor hypoxia-inducible factor-1 (HIF-1) in mediating these responses. Current work is focused on the regulation of HIF in the chronically hypoxic lung and evaluation of the potential for pharmacological inhibitors of HIF to prevent, reverse, or slow the progression of pulmonary hypertension.

hypoxia-inducible factor-1; pulmonary hypertension; chronic hypoxia

THE LUNG IS A UNIQUE ORGAN in many respects. The pulmonary circulation is the only vasculature required to accommodate the entire cardiac output and does so at arterial pressures ~10 times lower than the systemic circulation. The pulmonary vasculature is also unique in its response to hypoxia. In contrast to the systemic circulation, which dilates with hypoxia in an attempt to increase oxygen delivery to meet the metabolic demands of tissues, the pulmonary vessels constrict as oxygen tension falls. The first demonstration of this phenomenon can be attributed to Beutner [(7) and reviewed in (75)], working in the lab of Henry Pickering Bowditch’s mentor, Carl Ludwig. In 1946, the first detailed study characterizing this response in the intact cat was published by von Euler and Liljestrand (81). Although the precise teleology of hypoxic pulmonary vasoconstriction remains an area of debate, it is widely held that when the hypoxic challenge is short in duration and localized, as can happen with a small embolism or pneumonia, the vasoconstriction serves to divert blood from oxygen-poor areas of the lung to optimize ventilation/perfusion matching. However, when the hypoxic stimulus is global and prolonged, as can occur with residence at high altitude or many lung diseases, widespread vasoconstriction results in increased pulmonary vascular resistance, elevated pulmonary arterial pressure, and when severe and long enough in duration, eventual right heart failure. Although many of the structural and functional changes that occur in the lung with exposure to prolonged hypoxia have been documented, the mechanisms underlying the pathogenesis of hypoxic pulmonary hypertension remain incompletely understood. The following sections will focus on our work identifying some of the changes in pulmonary arterial smooth muscle cells (PASMCs) that occur in response to chronic hypoxia (CH), the role of the transcription factor, hypoxia-inducible factor 1 (HIF-1), in mediating these changes and recent work describing the regulation of HIF-1 in the hypoxic lung.

CHRONIC HYPOXIA AND THE LUNG

There are numerous instances, both physiological and pathological, during which the lung experiences prolonged exposure to localized or global hypoxia. For example, during embryogenesis, lung development occurs in a hypoxic environment (27). With respect to pathological conditions, prolonged alveolar hypoxia is a consequence of chronic lung diseases such as emphysema, chronic bronchitis, and cystic fibrosis and results in the development of pulmonary hypertension, which can have a deleterious effect on patient mortality and morbidity with the potential for eventual right heart failure. With current treatment modalities limited primarily to supplemental oxygen, mechanical ventilation, and lung transplant, the development of new therapeutic approaches to prevent and/or reverse pulmonary hypertension requires an understanding of the cellular mechanisms underlying pulmonary vascular responses to CH.

To explore the impact of long-term hypoxic exposure on the pulmonary circulation, investigators have developed and utilized animal models, in particular rats or mice exposed to normobaric or hypobaric hypoxia (\(P_{O_2} = 10%\) \(O_2\)) for 14–28 days in an environmental chamber. By using these rodent models, investigators have shown that pulmonary hypertension is attributable to both pulmonary vascular remodeling, characterized by smooth muscle cell proliferation, intimal thickening, and extension of smooth muscle into previously nonmuscular...
arterioles (10, 39, 71, 72, 80), and active contraction of vascular smooth muscle (21, 43, 48). Despite considerable advances in knowledge regarding the structural and functional changes that occur in the pulmonary vasculature in response to CH, the cellular mechanisms underlying the PASMC contraction, migration, hypertrophy, and hyperplasia that characterize pulmonary hypertension remain poorly understood.

**ABNORMALITIES IN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS**

Although it is clear that the pathogenesis of hypoxia-induced pulmonary hypertension involves changes in circulating factors and hemodynamic forces, an abundance of data has accumulated demonstrating that both the sustained vasoconstriction and vascular remodeling associated with CH may be related to abnormalities in the PASMCs. In particular, a number of studies now suggest that changes in PASMC function may be related to changes in membrane transporter/channel expression and intracellular ion concentrations, with our lab and others reporting hypoxia-induced changes in K⁺ channels, Ca²⁺ channels, and pH regulation.

**Effect of Chronic Hypoxia on K⁺ Channels**

K⁺ channels are the primary regulators of resting membrane potential (Eₘ) in PASMCs (3, 96). In turn, Eₘ controls the activity of sarcolemmal voltage-dependent Ca²⁺ channels (VDCC) and, thus, calcium influx and intracellular Ca²⁺ homeostasis. Changes in intracellular calcium concentration ([Ca²⁺]ᵢ) are critical for modulating PASMC function, with increased [Ca²⁺]ᵢ required for PASMC contraction (37, 59, 60), proliferation (12, 24, 74), and migration (28). PASMCs express a variety of K⁺ channel family subtypes; however, in adult animals under normal conditions, members of the voltage-gated K⁺ (Kᵥ) channel family bear most of the responsibility for controlling basal Eₘ. Inhibition of Kᵥ channels leads to membrane depolarization, activation of VDCC, and increased [Ca²⁺]ᵢ, (3, 96), and initial experiments reported that PASMCs isolated from rats exposed to CH exhibited depolarization (73) and reduced Kᵥ channel activity (68), suggesting that prolonged exposure to hypoxia altered K⁺ channel regulation and/or expression. Given that the effects of hypoxia were maintained for several hours after return to normoxic conditions, coupled with the fact that most adaptations to CH are presumably secondary to decreased Kᵥ channel activity, gave rise to the hypothesis that increased [Ca²⁺]ᵢ, attributable to activation of VDCC was the mechanism underlying hypoxic pulmonary hypertension (68, 73). However, this supposition was not supported by in vivo data, which demonstrated that regulation of Kᵥ channel expression. This hypothesis was first tested in vitro, where culture of PASMCs under hypoxic conditions for 3 days increased expression of mRNAs encoding several Kᵥ channel α (pore-forming)-subunits, including Kᵥ1.1, Kᵥ1.5, and Kᵥ2.1 (82). These results suggested that hypoxia could directly repress K⁺ channel expression; however, it could also be argued that the effect of short-term hypoxia in cultured cells may not accurately reflect the effects of CH on K⁺ channel expression in the intact animal, where the duration of exposure and level of hypoxia are likely to be different and where changes in hemodynamic stresses and/or locally produced or circulating factors may alter the response. To address this possibility, intact animal models of CH were used to test the effect of CH on pulmonary vascular smooth muscle Kᵥ channel expression in vivo (Fig. 1). The results from these studies (15, 52, 85) were similar to those obtained in vitro, suggesting that the reductions in Kᵥ current density observed in cultured cells may not accurately reflect the effects of CH on Kᵥ channel expression in the intact animal, where the duration of exposure and level of hypoxia are likely to be different and where changes in hemodynamic stresses and/or locally produced or circulating factors may alter the response.

Along with the pore-forming α-subunits, PASMCs also express several regulatory β-subunits that associate with α-subunits and exert an inhibitory influence by accelerating inactivation and/or shifting the voltage sensitivity of the α-subunits. Expression of inhibitory Kᵥ β-subunits was unaffected by in vitro (82) or in vivo (85) hypoxic exposure, suggesting that a reduction in Kᵥ channel number coupled with a maintained levels of inhibitory β-subunits (thereby increasing α- and β-subunit interactions) is the likely underlying cause of reduced Kᵥ current density. In stark contrast to the effects of CH on Kᵥ channel expression observed in pulmonary arteries, CH had no effect on Kᵥ channel expression in aortas from these animals (85), indicating that the regulation of Kᵥ channel expression by CH is a pulmonary-specific response. The mechanisms underlying differential effects of CH on pulmonary and systemic smooth muscle are still under investigation.

**Effect of Chronic Hypoxia on Ca²⁺ Homeostasis**

Alterations in Ca²⁺ homeostasis are associated with both hypoxic pulmonary vasoconstriction and PASMC proliferation and migration (12, 24, 28, 37, 59, 60, 74, 87). The finding that PASMCs from chronically hypoxic animals were depolarized, presumably secondary to decreased Kᵥ channel activity, gave rise to the hypothesis that increased [Ca²⁺]ᵢ, attributable to activation of VDCC was the mechanism underlying hypoxic pulmonary hypertension (68, 73). However, this supposition was not supported by in vivo data, which demonstrated that...
voltage-gated Ca\(^{2+}\) channel antagonists did not prevent development of hypoxia-associated pulmonary hypertension (20), or by clinical data showing that acute administration of vasodilators (41) but not Ca\(^{2+}\) channel antagonists (20), reduced pulmonary artery pressure in patients with hypoxic pulmonary hypertension attributable to chronic obstructive pulmonary disease. In attempting to answer whether activation of VDCCs was a driving force in the development and progression of hypoxic pulmonary hypertension, we initially investigated the effects of CH on Ca\(^{2+}\) homeostasis in PASMCs (67), demonstrating that basal [Ca\(^{2+}\)]\(_i\) was increased and confirming profound changes in Ca\(^{2+}\) regulation. That this increase in [Ca\(^{2+}\)]\(_i\) was rapidly normalized by removal of extracellular Ca\(^{2+}\) was not surprising and supported the hypothesis that active Ca\(^{2+}\) entry was required to maintain elevated [Ca\(^{2+}\)]\(_i\). However, the finding that VDCC blockers did not alter resting [Ca\(^{2+}\)]\(_i\) (67) was unexpected and ruled out influx through voltage-dependent enhancement of Ca\(^{2+}\) channel activity during CH. Similar findings came from isolated vessels studies, where removal of extracellular Ca\(^{2+}\) relaxed arteries from chronically hypoxic rats, whereas blockade of VDCCs had no effect on tone (67). Although these data confirmed that the increase in resting [Ca\(^{2+}\)]\(_i\) maintained sustained contraction of the pulmonary arteries during CH, they also eliminated a role for VDCCs.

Ca\(^{2+}\) influx in PASMCs can also occur via nonselective cation channels (NSCCs), which include a large family of proteins that appear to encode for both receptor-operated Ca\(^{2+}\) channels and store-operated Ca\(^{2+}\) channels. NSCCs are not activated by depolarization; rather, receptor-operated channels are activated by ligand binding to membrane receptors whereas store-operated Ca\(^{2+}\) channels are activated by depletion of intracellular stores. A main function of store-operated Ca\(^{2+}\) influx, or capacitative Ca\(^{2+}\) entry (CCE), is to replenish endoplasmic reticulum/sarcoplasmic reticulum stores. Functional studies have revealed that CCE is present in PASMCs (12, 83) and is involved in PASMC contraction and growth (12, 74). We (84) and others (29) found that CCE is greater in PASMCs from chronically hypoxic rats compared with PASMCs from normoxic rats and contributes to the maintenance of basal [Ca\(^{2+}\)]\(_i\) during CH because inhibitors of these channels decreased resting [Ca\(^{2+}\)]\(_i\) in PASMCs from hypoxic, but not normoxic, animals (Fig. 2).

Enhanced CCE following exposure to CH is attributable to increased expression of Ca\(^{2+}\)-permeable NSCCs, which are believed to be comprised of mammalian homologs of transient receptor potential (TRP) proteins, particularly isoforms in the canonical TRP (TRPC) subfamily, alone or in a complex with Orai1 and stromal interacting protein 1 (STIM1) proteins. Several of these proteins are known to be expressed in PASMCs (12, 29, 31, 83, 84), and experiments using RNA interference techniques have revealed that TRPC1, TRPC6, STIM1, and Orai1 all contribute to CCE in PASMCs (30, 45, 95). Examination of the levels of TRPC proteins in pulmonary vascular smooth muscle from normoxic and chronically hypoxic rats revealed that the expression of TRPC1 and TRPC6, but not TRPC4, increased in both the rat and murine models of hypoxic pulmonary hypertension (84).

To determine whether the effect of CH exposure on TRPC expression could be attributable to stimuli other than hypoxia (i.e., increased mechanical forces or altered exposure to circulating factors), PASMCs isolated from normoxic rats were cultured under hypoxic conditions (4% O\(_2\); 60 h). PASMCs exposed to hypoxia ex vivo also exhibited increased TRPC6 mRNA and protein levels and an increase in basal [Ca\(^{2+}\)]\(_i\) (84), indicating that CH upregulated TRPC expression through a direct effect on mRNA expression in PASMCs.

**Effect of Chronic Hypoxia on Intracellular pH**

Mammalian systems possess three primary mechanisms for maintaining intracellular pH (pH\(_i\)) homeostasis: the Na\(^+\)-dependent Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange, Na\(^+\)-independent Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange and Na\(^+\)/H\(^+\) exchange (NHE). All of these exchangers are present in vascular smooth muscle (32, 55), with NHE appearing to be the main mechanism responsible for regulating PASMC pH\(_i\) (55). The Na\(^+\)/H\(^+\) exchanger is a plasmalemmal protein that uses the transmembrane Na\(^+\) gradient to extrude protons. Tight control of NHE and pH\(_i\) is critical for maintaining cell viability, volume regulation, and mediator release. Early studies indicated that an increase in pH\(_i\) occurs during exposure of PASMCs to acute hypoxia (32) and during sustained hypoxic contraction (25). The importance of NHE in regulating cell function was underscored by studies showing

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*Fig. 2. Effect of nonselective cation channel inhibitors on resting intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) in pulmonary arterial smooth muscle cells from normoxic and chronically hypoxic rats. Data were collected from 10–30 cells per field and averaged to obtain a single value per experiment; each experiment was conducted on cells from different animals. [Ca\(^{2+}\)]\(_i\) levels were measured using fluorescent microscopy in Fura-2-loaded cells before (control) and 15 min after exposure to the nonselective cation channel inhibitors: A, SKF96365 (50 μM); B, NiCl\(_2\) (500 μM). n = 3 or 4 animals per condition. *Significantly different from normoxic control; †significantly from hypoxic control. Data modified with permission from Wang et al. (84).*
that activation of NHE and alkalization were required for growth factor-induced PASMC proliferation (53) and that hypoxia-induced pulmonary vascular remodeling was prevented by inhibitors of NHE (54).

In our initial studies examining the effects of CH on pH homeostasis, we found that basal pH$_{i}$ and NHE activity were elevated in PASMCs from chronically hypoxic animals (58). Because inhibitors of NHE reduced basal pH$_{i}$ in PASMCs from chronically hypoxic animals to near normal levels, we concluded that the alkaline shift in pH$_{i}$ was attributable to increased NHE activity. The fact that the change in pH$_{i}$ and NHE activity in PASMCs isolated from chronically hypoxic mice was observed for several days after returning to normoxic conditions suggested that the increase in NHE activity most likely resulted from an increase in NHE protein expression. Thus we examined the effect of CH on NHE expression in pulmonary vascular smooth muscle. Ten genes have been identified that encode different isoforms of the Na$^{+}$/H$^{+}$ exchanger (NHE1–10). NHEs 1–3 have been the most widely studied, with NHE1 ubiquitously expressed and NHE2 and NHE3 found predominately in the gastrointestinal epithelium, although low-level expression of NHE2 in the lung has been reported (9, 86). Little is known about the function and localization of NHE4 and NHE5, neither of which are present in the lung (4, 49) and NHE6–10 are thought to be localized in mitochondria, other organelles, or nonpulmonary tissues (14, 40, 44, 46, 47). By using RT-PCR and immunoblot, we demonstrated the presence of NHE1, but not NHE2 or NHE3, in mouse pulmonary vascular smooth muscle (58, 65). Examination of tissue from chronically hypoxic mice revealed that NHE1 mRNA and protein expression were increased significantly, correlating with the effect of CH on NHE activity and basal pH$_{i}$ (58). A recent study using mice deficient for NHE1 revealed that these animals exhibited reduced pulmonary vascular remodeling and decreased pulmonary hypertension in response to CH (94), confirming the importance of NHE1 in pulmonary vascular changes induced by CH. These data indicate that CH has dramatic effects on PASMC function and pH homeostasis via induction of NHE1.

ROLE OF HYPOXIA-INDUCIBLE FACTOR

After demonstrating that induction of NHE1 and TRPC proteins and downregulation of Kv channel expression during CH occurred at the level of transcription, we began to search for mechanisms that would mediate these responses. The hypoxia-inducible factors (HIFs), a family of oxygen-sensitive transcription factors, have been identified as critical mediators of adaptive responses to hypoxia, regulating the expression of dozens of genes important in growth, vascular development, and metabolism. The initial family member, hypoxia-inducible factor 1 (HIF-1), was identified as a basic helix-loop-helix protein bound to the hypoxia response element of the EPO gene during hypoxia (63). HIF-1 is highly conserved, tightly regulated by O$_{2}$ availability, found in all nucleated cells, and has been demonstrated to regulate the expression of hundreds of genes (61, 62). HIF-1 is a heterodimer, composed of HIF-1$\alpha$, which is found at very low levels under normoxic conditions, and HIF-1$\beta$, which is ubiquitously expressed. Thus it is the HIF-1$\alpha$ subunit that confers sensitivity and specificity for hypoxic induction of HIF-1 transcriptional activity.

The mechanism by which cells sense and transduce a drop in oxygen into increased HIF-1 activity was discovered in 2001 (Fig. 3) when several groups reported that HIF-1$\alpha$ ubiquitination required hydroxylation at two proline residues by prolyl hydroxylase domain (PHDs) proteins using molecular O$_{2}$ as a substrate (11, 17, 19, 34, 93). As O$_{2}$ levels decrease, PHD activity is reduced, hydroxylation at the proline residues ceases, and the protein is stabilized and translocates into the nucleus, where it binds HIF-1$\beta$ and recruits coactivator proteins to the HIF binding site within the hypoxia response element. To date, four PHD isoforms have been identified, although only PHD1–3 appear to hydroxylate HIF-1, with evidence suggesting that PHD2 is the primary isoform responsible for HIF-1$\alpha$ hydroxylation in vivo (2, 6, 38). In general, HIF-1$\alpha$ protein accumulation correlates with increased transcriptional activity; however, transactivation of HIF-1 is regulated by factor inhibiting HIF-1 (FIH-1), which hydroxylates HIF-1$\alpha$ at an asparagine residue within the COOH-terminal transactivation domain and prevents binding of the transcriptional coactivators CBP and p300 (33).

Regulation of Hypoxia-Inducible Factor in the Lung

As described in the preceding sections, we and others have shown that CH induces changes in PASMC ion channel/transporter expression. In the search for a possible mediator of hypoxic regulation of these genes, the transcription factor, HIF-1, became a leading candidate. Regulation of HIF-1$\alpha$ expression occurs at several levels. In mouse lung, HIF-1$\alpha$ mRNA is rapidly increased within 30 min of exposure to 7% oxygen (89). The oxygen-dependence of HIF-1$\alpha$ protein expression was initially demonstrated in an isolated, perfused ferret lung preparation, where oxygen levels could be precisely controlled and in the in vitro setting where acute exposure of cultured PASMCs and pulmonary endothelial cells to hypoxia increased HIF-1$\alpha$ protein levels (91). Accumulation of HIF-1$\alpha$ protein under these conditions was correlated with enhanced DNA binding activity, demonstrating both induction and activation of HIF-1.

In addition to hypoxia, HIF-1 can be activated by several physiological stimuli, including transforming growth factor-$\beta$, insulin, hydralazine, epidermal growth factor, platelet-derived growth factor, and angiotensin II (23, 36, 50, 57, 64, 77, 78, 97). Our studies have unexpectedly revealed an exciting new paradigm whereby the vasoconstrictor peptide, endothelin-1 (ET-1), itself a HIF target, regulates HIF-1 expression, leading to a positive feedback mechanism. Exposing PASMCs to exogenous ET-1, which mimics the release of ET-1 from endothelial cells, induced HIF-1$\alpha$ in the absence of hypoxia (51), findings that are in line with recent results in ovarian cancer and melanoma cells (69, 70). Moreover, we found that at moderate levels of hypoxia, which are at or near the threshold for HIF-1$\alpha$ induction (91) and are similar to those occurring in the pulmonary circulation with an FIO$_{2}$ of 10% (76), ET-1 is required for accumulation of HIF-1$\alpha$ in PASMCs, because upregulation of HIF-1$\alpha$ was prevented by ET-1 subtype A (ET$_{A}$) receptor antagonists (51). This feedforward mechanism of HIF-1 induction by ET-1 does not appear to be a general feature of all cells, because aortic smooth muscle cells do not exhibit HIF-1 induction in response to either moderate hypoxia or ET-1, raising the tantalizing possibility that the presence of this mechanism in PASMCs underlies the different functional response to hypoxia observed between these cell types.
Hypoxia Inducible Factor-1 and Ion Channels/Transporters

To evaluate the in vivo role of HIF-1, Iyer et al. (18) generated mouse embryonic stem cells homozygous or heterozygous for a null allele at the Hif1a locus exhibiting complete (Hif1a<sup>−/−</sup>) and partial deficiency (Hif1a<sup>+/−</sup>) for HIF-1α, respectively, that were subsequently used to generate transgenic Hif1a<sup>−/−</sup>/Hif1a<sup>−/−</sup> and Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> mice. Hif1a<sup>−/−</sup>/Hif1a<sup>−/−</sup> embryos died midgestation, whereas Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> mice were viable and phenotypically indistinguishable from their wild-type (Hif1a<sup>+/+</sup>/Hif1a<sup>+/+</sup>) littermates (18). Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> mice exposed to 10% O<sub>2</sub> for 3 wk exhibit right heart hypertrophy, elevated pulmonary artery pressure, polycythemia, and vascular remodeling (Fig. 4); however, these changes were markedly attenuated in chronically hypoxic Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> mice (92), demonstrating that HIF-1α plays a pivotal role in development of hypoxic pulmonary hypertension. We then utilized Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> and Hif1a<sup>+/−</sup>/Hif1a<sup>−/−</sup> mice to evaluate the role of HIF-1 in mediating the effect of CH on ion channels/transporters. With respect to Kv channels, decreased Kv current and channel expression, and concomitant depolarization were observed in PASMCs isolated from Hif1a<sup>−/−</sup> mice, whereas the effects of CH were reduced or absent in PASMCs from Hif1a<sup>+/−</sup> mice (66, 88), indicating that full expression of HIF-1α was required for the hypoxia-induced reduction in PASMC Kv channel activity. That HIF activation mediated repression of Kv channels was further demonstrated by the finding that overexpression of HIF-1 under normoxic conditions using an adenovirus that encodes a constitutively active form of HIF-1α (22) was able to cause downregulation of Kv1.5 and Kv2.1 expression (88).

The transcriptional regulation of TRPCs and NHEs is just beginning to be explored and much is yet to be learned with respect to the factors involved in the process. Because the genes encoding TRPC1, TRPC6, and NHE1 all contain putative HIF-1 binding sites, and previous work demonstrated a crucial role for HIF-1 in the pathogenesis of hypoxic pulmonary hypertension (92), we hypothesized that HIF-1 might be involved in the hypoxic induction of TRPC and NHE1 proteins. As anticipated based on data from chronically hypoxic rats, exposure to CH markedly increased TRPC1, TRPC6, and NHE1 expression in endothelium-denuded pulmonary arteries isolated from Hif1a<sup>−/−</sup> mice (65, 84). Functionally, PASMCs isolated from these animals displayed elevated [Ca<sup>2+</sup>]<sub>i</sub>, an alkaline shift in pH<sub>i</sub>, and increased NHE activity. In mice with
A specific PO2) or hypoxic (4% O2; 5% CO2) conditions for 72 h. BrDU was added for an experiment. Cells (5,000/well) were plated in basal media (Ham’s F-12 with 0.5% serum) and exposed to control (20% O2; 5% CO2) or hypoxic (4% O2; 5% CO2) conditions. Following expression of a constitutively active form of HIF-1α, TRPC1, TRPC6, and NHE1 expression were increased, as were resting pHΔ and NHE activity (65, 84).

Taken together, these data from loss-of-function and gain-of-function models provided strong evidence that HIF-1 was both necessary and sufficient for hypoxia-induced alterations in Kv, TRPC, and NHE1 proteins and indicated that HIF-1 plays a critical role in the hypoxic regulation of K+, Ca2+, and pHΔ homeostasis during CH (Fig. 5). Although all of the genes encoding these ion channels/transporters contain putative HIF binding sites, it remains to be determined 1) whether HIF-1 directly binds to the genes encoding these channels/transporters to regulate the induction/repression observed with CH; 2) whether other HIF-dependent intermediates may be involved; and 3) whether these changes are regulated independently or if alterations in the expression/activity of one channel can then modulate the expression of others.

Ongoing Experiments: Treatments Targeting Hypoxia Inducible Factor

Given their wide distribution throughout the body and the general lack of isoform-selective inhibitors, targeting TRPC1/6 partial HIF-1α deficiency, the effects of CH on basal [Ca2+], pHΔ and NHE activity and the hypoxic induction of TRPC1, TRPC6, and NHE1 were absent (84). To verify that the hypoxia-induced increase in protein expression was attributable to activation of HIF-1 and not an unrelated aspect of hypoxic exposure, HIF-1α was overexpressed in rat PASMCs isolated from normoxic animals and cultured under nonhypoxic conditions. Following expression of a constitutively active form of HIF-1α, TRPC1, TRPC6, and NHE1 expression were increased, as were resting pHΔ and NHE activity (65, 84).

Fig. 4. Reduced vascular remodeling in CH mice with partial deficiency for HIF-1α (Hfαα+/−). Mice were exposed to room air or FIO2 = 10% O2 for 3 wk. A: representative images show left lung sections stained for smooth muscle specific α-actin (SMA; brown) and counterstained with hematoxylin and eosin. Images are of vessels negative (left) and positive (right) for SMA. B: bar graph shows means ± SE data for the number of SMA-positive small diameter (<100 µm) vessels as a percentage of the total vessels counted per lung in normoxic (N) and CH wild-type (Hfαα+/+) and Hfαα−/− mice. n = 3 or 4 lungs per group. C: bar graph shows means ± SE data for PASMC proliferation measured using an ELISA for BrDU incorporation. Cells (5,000/well) isolated from normoxic Hfαα+/+ and Hfαα−/− mice were plated in basal media (Ham’s F-12 with 0.5% serum) and exposed to control (20% O2; 5% CO2) or hypoxic (4% O2; 5% CO2) conditions for 72 h. BrDU was added for an additional 24 h and incorporation into proliferating cells measured via ELISA. Absorbance values were normalized to control Hfαα+/+ within an experiment. n = 3 or 4 animals for each condition.

Fig. 5. Schematic detailing the effects of CH on pulmonary arterial smooth muscle cell ion homeostasis. Exposure to CH induces HIF-1, which leads to upregulation of Na+/H+ exchange isoform 1 (NHE1) and transient receptor potential canonical family member 1 (TRPC1) and downregulation of voltage-gated K+ channel family member 1.5 (Kv1.5). Alterations in channel/transporter expression cause depolarization and increased intracellular K+ concentration ([K+]i), an alkaline shift in intracellular pH (pHΔ), and elevated intracellular calcium concentration ([Ca2+]i).
or NHE1 for inhibition as a treatment for pulmonary hypertension was not a clinically reasonable approach. In contrast, HIF-1 is generally expressed at very low levels in adult tissues and only increases under certain conditions, providing an attractive target for therapeutic intervention. Until recently, inhibiting HIF-1 was a daunting task; however, in 2008, screening of 3,120 clinically used compounds in the Johns Hopkins Drug Library revealed several drugs that inhibit HIF-1α (98), including 11 cardiac glycosides. Digoxin, which has been used for decades as an inotrope to treat heart failure, was found to inhibit HIF-1α protein translation and blocked HIF-1 activity in vivo (90, 98). In chronically hypoxic mice receiving daily injections of digoxin, we found that the changes in pulmonary vascular [Ca\(^{2+}\)], pH, remodeling, and pressure (Fig. 6) were absent (1). Similar effects were observed with acriflavine (1), an antiseptic that prevents HIF-1α/HIF-1β dimerization (26). When digoxin treatment was initiated after pulmonary hypertension was established, right ventricular systolic pressure was reduced and Ca\(^{2+}\) and pH homeostasis was normalized (1). These data provide further evidence that HIF-1 plays a critical role in the development of hypoxia-induced pulmonary hypertension and demonstrate the ability of digoxin to slow the progression of the process. That elevated levels of HIF-1α and similar defects in PASMC function were observed in the lung of patients with other forms of pulmonary hypertension (8, 16, 42, 79) raises the possibility that digoxin, or other HIF inhibitors, might reduce pulmonary vascular pressure and remodeling in pulmonary hypertension not associated with hypoxia. Although digoxin has been proposed to increase cardiac contractility and output in patients with right ventricular failure (35, 56), the use of this drug in the pulmonary hypertension population remains controversial on the basis of the small therapeutic window, potential toxicity in the COPD patient population (5, 13), and a lack of data supporting a positive survival effect. Nonetheless, these data provide “proof of concept” of the potential beneficial effects of HIF-1 inhibitors and provide a starting point for further investigation.

CONCLUSIONS

Over the past two decades, we have endeavored to better understand the molecular signals that result in enhanced pulmonary arterial smooth muscle cell contraction, proliferation, and migration during the development of hypoxic pulmonary hypertension, with the hope that the information obtained could be used to interrogate novel therapeutic strategies. Relating the alterations in pulmonary arterial smooth muscle cell ion homeostasis that occur in chronic hypoxia models to the mechanistic underpinnings of different forms and severities of human pulmonary hypertension continues to pose a significant challenge. However, in a disease where treatment options are limited, our studies have pointed to possible cellular mechanisms involved in the pathogenesis of pulmonary hypertension, and although questions remain as to whether inhibitors of hypoxia-inducible factors will ultimately prove clinically beneficial in pulmonary hypertension patients, recent findings have demonstrated that old drugs can be taught new tricks. Clearly, much work is yet to be done, which will most certainly keep us occupied for the next two decades and beyond.

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No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

Author contributions: L.A.S. conception and design of research; L.A.S. performed experiments; L.A.S. analyzed data; L.A.S. interpreted results of experiments; L.A.S. prepared figures; L.A.S. drafted manuscript; L.A.S. edited and revised manuscript; L.A.S. approved final version of manuscript.

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