HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes

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1Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University School of Medicine; 2Institute for Cell Engineering, McKusick-Nathans Institute of Genetic Medicine, and Departments of Pediatrics, Medicine, Oncology, and Radiation Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland

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Shimoda, Larissa A., Michele Fallon, Sarah Pisarcik, Jian Wang, and Gregg L. Semenza. HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. Am J Physiol Lung Cell Mol Physiol 291: L941–L949, 2006. —Vascular remodeling resulting from altered pulmonary arterial smooth muscle cell (PASMC) growth is a contributing factor to the pathogenesis of hypoxic pulmonary hypertension. PASMC growth requires an alkaline shift in intracellular pH (pHi) and we previously showed that PASMCs isolated from mice exposed to chronic hypoxia exhibited increased Na+/H+ exchanger (NHE) expression and activity, which resulted in increased pHi. However, the mechanism by which hypoxia caused these changes was unknown. In this study we tested the hypothesis that hypoxia-induced changes in PASMC pHi homeostasis are mediated by the transcriptional regulator hypoxia-inducible factor 1 (HIF-1). Consistent with previous results, increased NHE isoform 1 (NHE1) mRNA and protein, enhanced NHE activity, and an alkaline shift in pHi, were observed in PASMCs isolated from wild-type mice exposed to chronic hypoxia (3 wk at 10% O2). In contrast, these changes were absent in PASMCs isolated from chronically hypoxic mice with partial deficiency for HIF-1. Exposure of PASMCs to hypoxia ex vivo (48 h at 4% O2) or overexpression of HIF-1 in the absence of hypoxia also increased NHE1 mRNA and protein expression. Our results indicate that full expression of HIF-1 is essential for hypoxic induction of NHE1 expression and changes in PASMC pHi homeostasis and suggest a novel mechanism by which HIF-1 mediates pulmonary vascular remodeling during the pathogenesis of hypoxic pulmonary hypertension.

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changes in PASMC membrane potential, K\(^+\) channel activity, and Ca\(^{2+}\) homeostasis were markedly attenuated or absent in
*Hif1a\(^{-/-}\)* mice exposed to CH (42, 48, 50). Although the promoter of the gene encoding NHE1 contains putative HIF-1-
binding sites, the regulation of NHE1 by HIF-1 has not been studied. In this study, we tested the hypothesis that hypoxic
induction of NHE1 expression and subsequent alterations in
pH homeostasis in PASMCs are mediated by HIF-1.

**METHODS**

*Chronic hypoxic exposure.* All procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University.
Adult male Wistar rats and C57BL/6 mice were placed in a hypoxic chamber for 21 days. The chamber was continuously flushed with a mixture of room air and N\(_2\) (10 \(\pm\) 5% O\(_2\)) to maintain low CO\(_2\) (<0.5%). Chamber O\(_2\) concentration was continuously monitored (PRO-OX; RCI Hudson, Anaheim, CA). The animals were
exposed to room air for 10 min twice a week to clean the cages and
replenish food and water supplies. At the end of hypoxic exposure,
animals were injected with heparin and anesthetized with pentobar-
bital sodium (43 mg/kg ip). The heart and lungs were removed en bloc and transferred to a petri dish of physiological salt solution (PSS) containing (in mM): 130 NaCl, 5 KCl, 1.2 MgCl\(_2\), 1.5 CaCl\(_2\), 10 HEPES, and 10 glucose, with pH adjusted to 7.2 with 5 M NaOH.
The right ventricle (RV) of the heart was separated from the left ventricle and the septum (LV+S) following removal of the atria, and the two portions were blotted dry and weighed.

*Cell isolation and culture.* The method for obtaining single
PASMCs has been described previously (39, 43). Briefly, intrapulmo-
nary arteries (200–500 \(\mu\)m OD) were isolated and cleaned of con-
nective tissue. After the endothelium was disrupted by gentle rubbing of the luminal surface with a cotton swab, the arteries were allowed to recover for 30 min in cold (4°C) PSS, followed by 20 min in reduced Ca\(^{2+}\) PSS (20 \(\mu\)M CaCl\(_2\)) at room temperature. The tissue was
digested in reduced Ca\(^{2+}\) PSS containing collagenase (type I, 1,750 U/ml), papain (9.5 U/ml), bovine serum albumin (2 mg/ml), and
dithiothreitol (DTT; 1 mM) at 37°C for 10 min (murine SMCs) or 25 min (rat PASMCs). Following digestion, single smooth muscle
cells were dispersed by gentle trituration with a wide-bore transfer pipette in Ca\(^{2+}\)-free PSS, and the cell suspension was placed on
25-mm glass coverslips for pHi measurements or grown to 80%
confluence in 6-cm culture dishes for protein and RNA. Murine PASMCs were cultured in SmBm Complete Media (Clonetics) supplemented with 10% fetal calf serum (FCS) for 3–4 days and placed in serum-free media 24 h before experiments. Rat PASMCs were cultured in Ham’s F-12 media supplemented with 0.5% FCS and 1%
penicillin/streptomycin. In some experiments, PASMCs were cultured under hypoxic conditions (4% O\(_2\), 5% CO\(_2\)) to maintain low
CO\(_2\) and hypoxic (4% O\(_2\)) conditions. *Significant difference from control
(row = 0.05).

**Intracellular pH measurements.** PASMCs were placed in a laminar flow cell chamber perfused with either Krebs bicarbonate solution containing (in mM): 118 NaCl, 4.7 KCl, 0.57 MgSO\(_4\), 1.18 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 2.5 CaCl\(_2\), and 10 glucose gassed with 16% O\(_2\), 5% CO\(_2\), or a HEPES-buffered PSS containing (in mM): 118.3 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 25 NaHCO\(_3\), 1.1 glucose, and 1.2 KH\(_2\)PO\(_4\). pHi was measured in cells incubated with a membrane-permeant (acetoxymethylether ester) form of the pH-sensitive fluorescent dye BCECF-AM for 60 min at 37°C under an atmosphere of 21% O\(_2\), 5% CO\(_2\), and then washed with PSS for 15 min at 37°C to remove extracellular dye and allow complete de-esterification of cytosolic dye. Ratiometric measurement of fluorescence from the dyes was performed on a Nikon TSE 100 Eclipse inverted microscope with epifluorescence attachments. The light beam from a xenon arc lamp was filtered by interference filters at 490 and 440 nm and focused onto the PASMCs
under examination via a \(\times20\) fluorescence objective (Super Fluor 20, Nikon). Light emitted from the cell at 530 nm was returned through the objective and detected by a cooled CCD imaging camera. An electronic shutter (Sutter Instruments) was used to minimize photo-
bleaching of dye. Protocols were executed and data were collected online with InCyte software (Intracellular Imaging). pHi was esti-
imated from in situ calibration after each experiment. Cells were perfused with a solution containing (in mM): 105 KCl, 1 MgCl\(_2\), 1.5 CaCl\(_2\), 10 glucose, 20 HEPES-Tris, and 0.01 nigericin to allow pHi to equilibrate to external pH. A two-point calibration was created from fluorescence measured as pHi was adjusted with KOH from 6.5 to 7.5.

Baseline pHi was measured in PASMCs for 3 min, and values were averaged to obtain a mean value for each cell. A standard ammonia pulse technique was used to measure Na\(^+\)/H\(^+\) exchange activity (Fig. 1A). PASMCs loaded with BCECF were placed on the fluorescence microscope and perfused at a rate of 2.5 ml/min with HEPES1 solution containing (in mM): 130 NaCl, 5 KCl, 1 MgCl\(_2\), 1.5 CaCl\(_2\), 10 glucose, and 20 HEPES with pH adjusted to 7.4 with NaOH at 37°C. Baseline pHi was measured for 2 min before cells were briefly exposed to NH\(_4\)Cl (ammonium pulse) by perfusing with HEPES2 solution containing (in mM): 110 NaCl, 20 NH\(_4\)Cl, 5 KCl, 1 MgCl\(_2\), 1.5 CaCl\(_2\), 10 glucose, and 20 HEPES at a pH of 7.4 using NaOH for 3 min. The ammonium pulse caused alkalization due to influx of NH\(_3\) and buffering of intracellular H\(^+\). Washout of NH\(_4\)Cl in the absence of extracellular Na\(^+\) using a Na\(^+\)- and NH\(_4\)\(^+\)-free solution containing (in mM): 130 choline chloride, 5 KCl, 1 MgCl\(_2\), 1.5 CaCl\(_2\), 10 glucose, and 20 HEPES at a pH of 7.4 using KOH for 10 min

**Fig. 1**.  A: representative trace of ammonium pulse technique used to monitor baseline pHi and recovery rate from acidosis in pulmonary arterial smooth muscle cells (PASMCs). Cells were maintained at control conditions for 2 min and then treated with NH\(_4\)Cl, which caused alkalization. A Na\(^+\)/NH\(_4\)Cl-free solution caused cellular acidification due to extrusion of NH\(_3\) while preventing exchange activity. Readdition of Na\(^+\) allowed exchange activity to resume. Recovery rates were measured by calculating the intracellular pH (pHi) change over a 2-min recovery period. B: representative traces demonstrating ammonia pulse and Na\(^+\)/H\(^+\) exchange activity in PASMCs cultured under control or hypoxic conditions. C and D: bar graphs illustrating average basal pHi (C) and Na\(^+\)/H\(^+\) exchange activity (D) measured in PASMCs cultured under control and hypoxic (n = 49 cells) conditions. *Significant difference from control
value (P < 0.05).
resulted in acidification due to rapid diffusion and washout of NH₃, leaving behind H⁺ ions. The external solution was then switched back to HEPES solution for 10 min. Readdition of extracellular Na⁺ allows activation of Na⁺/H⁺ exchange and recovery from acidification to basal levels. The rate of Na⁺-dependent recovery from intracellular acidification corresponds to Na⁺/H⁺ exchange activity.

**RT-PCR.** Total RNA was prepared from endothelium-denuded interobal vessels by TRIzol extraction. Two arteries each from three mice were combined per sample. Isolated total RNA was dissolved in diethylpyrocarbonate-treated water at 1 µg/µl and stored at −80°C until use. Reverse transcription was performed using the first-strand cDNA synthesis kit (Pharmacia Biotech). One microgram of the total RNA was reverse transcribed using random hexamers incubated for 1 h at 37°C. The reverse transcriptase was inactivated by heating the mixture for 5 min at 90°C. Specific primers for NHE1, NHE2, NHE3, and β-actin (Table 1) were designed from sequences of the coding regions corresponding to mouse and rat NHE1–3 and β-actin genes (2, 39). PCR was performed using the GeneAmp PCR system (Perkin-Elmer) using Taq polymerase. Three microliters of the first-strand cDNA mixture was amplified by annealing at 60°C for 1 min, extending at 72°C for 2 min, and denaturing at 94°C for 1 min. Preliminary experiments were performed to determine the optimum number of cycles (30 cycles). After 30 cycles, a final extension was run at 72°C for 10 min. PCR products were electrophoresed through a 1% agarose gel and stained with ethidium bromide for visualization under UV light.

**Immunoblot assay.** For each sample, cells from three animals were isolated and cultured in 60-mm petri dishes containing SmBm complete media supplemented with 10% FCS for 3–5 days and then serum-starved for 24 h before harvest. PASMCs were scraped and cells were lysed in 1 ml of cold lysis buffer containing (in mM): 25 HEPES, 1 DTT, and the protease inhibitor cocktail Complete tablets (Boehringer). Protein concentrations were calculated from a standard Bradford assay. For each sample, 100 µg of total protein were used and separated on 10% SDS-pag gels and transferred to nitrocellulose membranes. Membranes were blocked in 5% nonfat milk for 2 h and probed with primary antibody (Chemicon) overnight at 4°C, washed, and incubated in secondary antibody (BioRad) for 1 h. Bands were visualized by enhanced chemiluminescence according to the manufacturer’s instructions. Membranes were then stripped and reprobed for β-actin. Densitometry was performed to quantify the amount of protein, and the ratio of NHE1 to β-actin was calculated. Fold induction was determined by setting the ratio of NHE1/β-actin in normoxic animals equal to 1.

**Adenovirus infection.** PASMCs were cultured to 80% confluence and placed in serum-free media for 24 h. Cells were subjected to infection with replication-defective recombinant adenoviruses encoding either β-galactosidase (AdLacZ) or a constitutively active form of HIF-1α (AdCA5), which contains mutations that inhibit degradation of the protein under hypoxic conditions (18). PASMCs were inoculated with 50 plaque-forming units per cell and incubated for 48 h at 37°C under hypoxic conditions.

**Data analysis.** Data are expressed as means ± SE; n is the number of experiments performed, or the number of cells in each experiment, as indicated. Statistical comparisons were performed using Student’s t-test (paired or unpaired) or ANOVA, as appropriate. Differences were considered to be significant when P < 0.05.

**RESULTS.**

**Direct effect of hypoxia on pH homeostasis in PASMCs.** We previously demonstrated that exposure to CH in vivo resulted in an alkaline shift in PASMC pHi and increased NHE activity. However, in vivo, in addition to hypoxia, the vascular cells are also exposed to increased pressure and circulating factors that may influence cell function. To determine whether hypoxia exerted a direct effect on pH homeostasis, we examined the direct effect of hypoxia on pHi and Na⁺/H⁺ exchange activity in PASMCs isolated from normoxic rats and cultured under hypoxic conditions ex vivo. To isolate the change in pHi, due to hypoxia-induced alterations in Na⁺/H⁺ exchange activity, PASMCs were superfused with bicarbonate-free (HEPES-buff ered) extracellular solution, which eliminates contributions from the Cl⁻/HCO₃⁻ exchangers. Under these conditions, basal pHi was significantly greater in PASMCs exposed to 4% O₂ for 48 h (6.98 ± 0.02; n = 66 cells) compared with cells cultured under nonhypoxic conditions (6.70 ± 0.03; n = 63 cells; Fig. 1C). Na⁺/H⁺ exchange activity, measured using the ammonium pulse technique (Fig. 1B), was also greater in PASMCs cultured under hypoxic conditions (0.096 ± 0.01 pH U/min in 56 cells vs. 0.18 ± 0.01 pH U/min in 49 cells; Fig. 1D), indicating that hypoxia directly alters pH homeostasis in PASMCs, independent of changes in arterial pressure or circulating factors.

**Direct effect of hypoxia on NHE1 expression in PASMCs.** To determine whether the hypoxia-induced changes in pHi and Na⁺/H⁺ exchange activity observed in PASMCs cultured under hypoxic conditions resulted from alterations in NHE1 expression, mRNA and protein levels were measured. To date, nine NHE isoforms have been characterized. NHE1 is ubiquitously expressed, whereas NHE2 and NHE3 are found predominately in the kidney and gastrointestinal epithelium (3, 35, 47, 49), although low-level expression of both isoforms in the lung has been reported (3, 49). Expression of NHE4 and NHE5 is only expressed, whereas NHE2 and NHE3 are found predominately in the kidney and gastrointestinal epithelium (3, 35, 47, 49), although low-level expression of both isoforms in the lung has been reported (3, 49). Expression of NHE4 and NHE5 is restricted primarily to the gastrointestinal tract and brain, respectively (1, 33), and although very low expression of NHE6–9 has been reported in whole lung tissue, localization of these isoforms is restricted to subcellular organelles (10, 26, 28, 30, 31). Initial screening experiments using specific primers designed for rat NHE1, NHE2, and NHE3 were performed

<table>
<thead>
<tr>
<th>Table 1. Primers used for RT-PCR</th>
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<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>NHE1 (mouse)</td>
</tr>
<tr>
<td>NHE1 (rat)</td>
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<tr>
<td>NHE2</td>
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<td>NHE3</td>
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<td>β-Actin</td>
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NHE, Na⁺/H⁺ exchanger.
We next examined whether HIF-1 mediated the hypoxic.

Table 2. Means ± SE values for heart weight measurement in normoxic and chronically hypoxic Hif1a+/+ and Hif1a−/− mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>RV Weight, g</th>
<th>LV + S Weight, g</th>
<th>RV/LV + S Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic Hif1a+/+</td>
<td>0.0251±0.002</td>
<td>0.1050±0.005</td>
<td>0.239±0.01</td>
</tr>
<tr>
<td>Hypoxic Hif1a+/+</td>
<td>0.0332±0.002*</td>
<td>0.0988±0.004</td>
<td>0.335±0.009*</td>
</tr>
<tr>
<td>Normoxic Hif1a−/−</td>
<td>0.0267±0.002</td>
<td>0.1077±0.005</td>
<td>0.243±0.007</td>
</tr>
<tr>
<td>Hypoxic Hif1a−/−</td>
<td>0.0286±0.002*</td>
<td>0.0961±0.005</td>
<td>0.296±0.009*</td>
</tr>
</tbody>
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RV, right ventricle; LV + S, left ventricle + septum; n = number of animals; *significant difference from normoxic Hif1a+/+ value; †significant difference from normoxic Hif1a−/− value; ‡significant difference from hypoxic Hif1a+/+ value.

Fig. 2. A and B: representative images illustrating RT-PCR products for NHE isoform 1 (NHE1) mRNA expression in rat PASMCs and NHE1-3 mRNA expression in rat kidney. C. RT-PCR products indicating increased expression of NHE1 mRNA in PASMCs under hypoxic conditions compared with PASMCs cultured under nonhypoxic conditions. Hypoxia had no effect on β-actin mRNA expression. Bar graph shows mean fold induction in NHE1 mRNA expression normalized to β-actin expression in PASMCs cultured under hypoxic conditions. *Significant difference from control value (P < 0.05). D: representative immunoblot image demonstrating increased NHE1 protein expression in PASMCs cultured under hypoxic conditions. Bar graph shows mean fold induction in NHE1 protein expression in PASMCs cultured under hypoxic conditions. *Significant difference from control value (P < 0.05). Results shown are representative of those obtained in 3 separate experiments using cells derived from 3 different animals. C, control; H, hypoxic.
HIF-1 regulates pH homeostasis in PASMCs.

**Effect of CH on Na⁺/H⁺ exchange activity in PASMCs from Hif1α+/+ and Hif1α−/− mice.** Na⁺/H⁺ exchange activity was similar in PASMCs isolated from normoxic Hif1α+/+ and Hif1α−/− mice (0.104 ± 0.02 vs. 0.190 ± 0.01 pH U/min, respectively) (Fig. 3B). Consistent with our previous results, exposure to CH markedly increased Na⁺/H⁺ exchange activity in PASMCs isolated from Hif1α+/+ mice (0.156 ± 0.02 pH U/min). In contrast, the CH-induced increase in Na⁺/H⁺ exchange activity was absent in PASMCs isolated from mice partially deficient for HIF-1 (0.088 ± 0.01 pH U/min).

**Effect of CH on NHE1 expression in Hif1α+/+ and Hif1α−/− mice.** As anticipated, NHE1 mRNA expression was increased in endothelium-denuded pulmonary arteries isolated from Hif1α+/+ mice exposed to CH compared with mRNA levels in pulmonary arteries isolated from normoxic Hif1α+/+ (Fig. 4A). Immunoblot analysis revealed a similar increase in NHE1 protein expression in these animals (Fig. 4B). Neither mRNA nor protein expression of NHE1 increased in response to CH in pulmonary arteries from Hif1α−/− mice.

**Effect of overexpression of HIF-1α on NHE1 expression.** To verify that the hypoxia-induced increase in NHE1 expression was due 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. Previous studies in this cell type demonstrated that transfection with an adenovirus containing a construct encoding AdCA5 increased HIF-1α protein levels and HIF-1 target gene expression under nonhypoxic conditions (18). Following transfection with AdCA5 for 48 h, an increase in NHE1 mRNA expression was observed compared with PASMCs transfected with AdLacZ (Fig. 5A). A similar increase in NHE1 protein expression was observed in PASMCs transfected with AdCA5 compared with AdLacZ (Fig. 5B).

Transfection alone did not significantly impact basal pH𝑖 or Na⁺/H⁺ exchange activity. Basal pH𝑖 was similar in control PASMCs and those transfected with AdLacZ (6.71 ± 0.02; n = 77 cells). However, basal pH𝑖 was significantly increased in PASMCs transfected with AdCA5 (6.92 ± 0.02; n = 76 cells; Fig. 6, A and B). In addition, Na⁺/H⁺ exchange activity was significantly greater in PASMCs transfected with AdCA5 (0.177 ± 0.01; n = 76 cells) than in PASMCs transfected with AdLacZ (0.068 ± 0.06; n = 77 cells; Fig. 6C).

**DISCUSSION**

Previous studies demonstrated that the development of hypoxic pulmonary hypertension was associated with alterations in PASMC pH homeostasis, including an alkaline shift in basal pH𝑖 due to increased expression and activity of NHE1 (39). However, the mechanism responsible for these changes was not known. In the current study, we demonstrated that the changes in pH homeostasis observed in PASMCs isolated from chronically hypoxic animals were due to a direct effect of hypoxia and used loss-of-function and gain-of-function models to demonstrate a critical role for HIF-1 in regulating these responses.

The factors controlling transcriptional regulation of NHE1 are poorly understood. The promoter region contains putative binding sites for a variety of transcription factors, including HIF-1. We previously demonstrated that numerous pulmonary responses to hypoxia are regulated by this transcription factor, including elevated RV pressure (an estimate of pulmonary arterial pressure), decreased K⁺ channel activity, PASMC hypertrophy, alterations in Ca²⁺ homeostasis, and pulmonary vascular remodeling (42, 48, 50). In this study, we used mice with partial deficiency for HIF-1α to evaluate the role of HIF-1 in the changes in pH homeostasis induced by CH. Consistent with our previous results in C57BL/6 mice, an alkaline shift in pH𝑖, increased Na⁺/H⁺ exchange activity, and augmented NHE1 expression were observed in pulmonary vascular smooth muscle isolated from chronically hypoxic wild-type mice. These alterations were absent in Hif1α−/− mice, indicating that full HIF-1 activity was required for the alterations in pH homeostasis observed in response to chronic hypoxia in vivo.
In the intact animal, exposure to hypoxia results in increased pulmonary arterial pressure and alterations in circulating factors, which may influence the pulmonary vascular changes observed in this model (6, 9, 19). Some of the circulating factors, such as endothelin-1, are regulated by HIF-1 (13). Moreover, as we previously reported (50), measurement of right ventricular hypertrophy verified that development of pulmonary hypertension in response to CH was blunted in mice partially deficient for HIF-1. Thus it was impossible to determine whether the lack of changes in NHE1 expression, Na⁺/H⁺ exchange activity, and pHᵢ in chronically hypoxic Hif1a⁻/⁻ mice was a direct consequence of reduced HIF-1-dependent NHE1 gene transcription, simply a result of decreased pulmonary arterial pressure, or a difference in the circulating factors to which the PASMCs were exposed in vivo.

We sought to address this issue in two ways. First, to explore whether the changes in PASMC pH homeostasis were due to a direct effect of hypoxia on PASMCs, an indirect effect mediated by pressure, or hypoxia-induced factors secreted by endothelial cells, we used an ex vivo model to assess the ability of hypoxia to alter pHᵢ, Na⁺/H⁺ exchange activity, and NHE1 expression in pulmonary arteries from hypoxic Hif1a⁻/⁻ mice and normoxic and chronically hypoxic Hif1a⁻/⁻ mice (n = 3 samples consisting of cells isolated from 3 mice each).

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expression. We found that exposure of PASMCs to hypoxia ex vivo, in the absence of increased pulmonary arterial pressure or circulating factors, caused changes in pH and Na+/H+ exchange activity that were qualitatively and quantitatively similar to those observed in cells isolated from animals exposed to hypoxia in vivo. We also found that NHE1 gene and protein expression were significantly increased in these cells to levels similar to those measured in endothelium-denuded pulmonary arteries from chronically hypoxic animals. Interestingly, in rat PASMCs, NHE1 protein was present as a double band. Other investigators have observed similar patterns of protein expression, due to the presence of several glycosylation sites (29, 51). It is likely that the faster migrating band corresponds to newly synthesized, core-glycosylated NHE1, whereas the slower migrating band corresponds to mature (terminally glycosylated) NHE1 (4). The data from PASMCs exposed to hypoxia ex vivo provide confirmation that hypoxia induces PASMC-autono-

mous changes in pH homeostasis and NHE1 expression that are similar to those observed during development of hypoxic pulmonary hypertension.

We next used a gain-of-function model to determine the effect of increased HIF-1 expression in the absence of hypoxia. Overexpression of HIF-1α resulted in an increase in NHE1 gene and protein expression that was similar to that observed in PASMCs exposed to hypoxia ex vivo and in pulmonary arteries isolated from chronically hypoxic animals. The increase in NHE1 expression appears to have had a functional consequence as well, resulting in elevated basal pH and enhanced Na+/H+ exchange activity. These results indicate that forced expression of HIF-1α in the absence of hypoxia was sufficient to induce changes in NHE1 expression and confirm a direct role for HIF-1 in regulating pH homeostasis.

Studies have demonstrated that an alkaline shift in pH and activation of Na+/H+ exchange were required for PASMC proliferation in response to growth factors (37). Furthermore, treatment with Na+/H+ exchange inhibitors attenuated pulmonary vascular remodeling in chronically hypoxic rats (37, 38). We previously reported that pulmonary vascular remodeling during CH was attenuated in Hif1a+/− mice, with a reduction in the number of small muscular vessels, decreased wall thickness, and absent PASMC hypertrophy (42, 50). Given the critical role pH homeostasis plays in PASMC growth, it is likely that the loss of hypoxia-induced alkalization in PASMCs isolated from chronically hypoxic Hif1a+/− mice provides one mechanism by which remodeling was attenuated in this model. Additionally, we recently found that HIF-1 also regulates Ca2+ homeostasis in PASMCs from chronically hypoxic animals via upregulation of store-operated Ca2+ channels and enhanced Ca2+ influx (48). As is the case with pH, an increase in intracellular Ca2+ concentration is associated with PASMC proliferation (34, 46). When taken together, our data provide evidence that HIF-1 controls two important mechanisms that may act in concert to facilitate pulmonary vascular remodeling during the pathogenesis of hypoxic pulmonary hypertension.

In summary, we used in vivo loss-of-function and ex vivo gain-of-function models to demonstrate that HIF-1 plays a crucial role in controlling NHE1 expression, Na+/H+ exchange activity, and alkalization of PASMCs in response to hypoxia. Although the current study was restricted to PASMCs, HIF-1-dependent hypoxia-induced changes in pH homeostasis may also regulate proliferation and apoptosis in other cell types. For example, exposure of cardiac myocytes to hypoxia induces apoptosis that can be attenuated by NHE inhibitors (44), and inhibition of Na+/H+ exchange in tumor cells decreases cell proliferation (11). Further study will also be required to determine whether HIF-1-dependent hypoxic induction of NHE1 is restricted to PASMCs or occurs in other pulmonary vascular cell types (e.g., endothelial cells and fibroblasts) that proliferate in response to chronic hypoxia.

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