Hypoxemia and Hypertension

Loss of the Aryl Hydrocarbon Receptor Induces Hypoxemia, Endothelin-1, and Systemic Hypertension at Modest Altitude

Amie K. Lund, Larry N. Agbor, Nan Zhang, Amy Baker, Huawei Zhao, Gregory D. Fink, Nancy L. Kanagy, Mary K. Walker

Abstract—The aryl hydrocarbon receptor (AHR) is a basic helix-loop-helix Per-Arnt-Sim transcription factor that mediates induction of metabolic enzymes and toxicity of certain environmental pollutants. Although AHR knockout (KO) mice develop cardiac hypertrophy, conflicting reports associate this pathology with hypotension or endothelin (ET)-1–dependent hypertension. Because hypertension occurred at modest altitude, we tested the hypothesis that loss of AHR increases the sensitivity to hypoxia-induced ET-1, contributing to systemic hypertension. We found that AHR KO mice were hypertensive at modest altitude (1632 m) but hypotensive at low altitude (225 m). When AHR KO mice residing at 1632 m were exposed to the partial pressure of inspired oxygen (PIO₂) at sea level for 11 days, blood pressure declined to levels measured at 225 m. Although plasma ET-1 in AHR KO mice was significantly elevated at 1632 m and decreased at 225 m and sea level PIO₂, pulmonary prepro-ET-1 mRNA was significantly reduced at 1632 m and decreased further at 225 m and sea level PIO₂. Blood gas analysis revealed that AHR KO mice were hypoxicemic, hypercapnic, and acidotic at 1632 m, values that were attenuated and normalized after 24 hours and 11 days under sea level PIO₂, respectively. Lastly, AHR inactivation in endothelial cells by small interfering RNA significantly reduced basal prepro-ET-1 mRNA but did not alter hypoxia-induced expression. Our studies establish the AHR KO mouse as a model in which modest decreases in PIO₂ lead to hypoxemia, increased plasma ET-1, and systemic hypertension without increased pulmonary prepro-ET-1 mRNA expression. (Hypertension. 2008;51:803-809.)

Key Words: blood pressure ■ hypertension ■ endothelin ■ oxygen ■ gene regulation

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor belonging to the basic helix-loop-helix Per-Arnt-Sim family of DNA binding proteins, which also includes hypoxia-inducible factors.¹ Although the AHR is known to mediate induction of drug-metabolizing enzymes and toxicity after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin, recent evidence has revealed a physiological role for AHR in cardiovascular homeostasis. AHR knockout (KO) mice develop cardiac hypertrophy,²,³ which is mediated, in part, by elevated plasma angiotensin II and endothelin-1 (ET-1).⁴,⁵ There are conflicting reports, however, of whether the cardiac hypertrophy is associated with systemic hypertension. Lund et al⁴,⁵ reported that cardiac hypertrophy in AHR KO mice is preceded by hypertension, whereas Vasquez et al⁶ and Ichihara et al⁷ reported that AHR KO mice are hypotensive and normotensive, respectively. The explanation for the disparate blood pressure values among these studies is unclear.

AHR and hypoxia-inducible factor-1α share a common dimerization partner, AHR nuclear translocator (hypoxia-inducible factor-1β), as well as other transactivators, and studies have shown that these 2 signal transduction pathways can exhibit reciprocal inhibitory cross-talk.⁸,⁹ The mechanism by which this functional interference occurs is not clear, nor has the physiological relevance of these interactions been defined. If AHR functions physiologically to attenuate hypoxia-induced responses, then AHR KO mice might exhibit an increased sensitivity to hypoxia-mediated gene induction and changes in physiology. Evidence supporting this idea was published recently showing that AHR KO mice are more responsive to the induction of vascular endothelial growth factor and neovasculogenesis after hindlimb ischemia.⁷ Because hypoxia is a potent stimulus of the vasoconstricting peptide, ET-1,¹⁰,¹¹ and because ET-1–dependent hypertension was reported in AHR KO mice residing at a modest altitude (Albuquerque, NM, 1632 m), we reasoned that the differences in blood pressure reported in AHR KO mice may result from differences in how the mice respond to changes in the partial pressure of inspired oxygen (PIO₂). Thus, we tested...
the hypothesis that loss of AHR enhances hypoxia-induced ET-1 expression and increases blood pressure in animals exposed to modest hypoxia.

Methods
An expanded Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.

Animals
AHR KO mice were obtained from Dr Frank Gonzalez (National Cancer Institute, Bethesda, Md) and backcrossed 11 generations to C57Bl/6N, whereas AHR WT mice were purchased from Harlan at 6 to 8 weeks of age. All of the study protocols were reviewed and approved by the University of New Mexico Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Blood Pressure Analysis
Arterial blood pressure was measured using radiotelemetry (Data Sciences International). At the University of New Mexico, blood pressure was continuously recorded for 6 days at environmental PIO2 (122 mm Hg) and continued to be recorded when mice were exposed to simulated sea level PIO2 (150 mm Hg) for 11 days.

Analysis of Blood Gases, Hemoglobin, and Hematocrit
Arterial pO2, pCO2, oxygen saturation, pH, and total CO2 were measured with an i-STAT (Abbott Point of Care Inc) from a carotid blood sample taken 2 to 3 days after catheter implantation. Blood gases were analyzed in mice residing at 1632 m and after 24 hours or 11 days of exposure to simulated sea level PIO2. Venous hematocrit and hemoglobin were measured with the i-STAT from mice residing at 1632 m and after 11 days of exposure to simulated sea level PIO2.

Analysis of ET-1 Expression
ET-1 in plasma and cell culture media was assayed by radioimmunoassay (Amersham Pharmacia) and ELISA (R&D Systems), respectively. Pulmonary prepro-ET-1 mRNA was analyzed from total RNA using quantitative PCR with SYBR green detection, an Icycler assay (Amersham Pharmacia) and ELISA (R&D Systems), respectively. Pulmonary prepro-ET-1 mRNA was analyzed from total RNA using quantitative PCR with SYBR green detection, an Icycler normalization control.

Cell Culture Studies
Human umbilical vein endothelial cells and microvascular endothelial cells isolated from lung were purchased from Lonza. Cells were transfected with control or AHR small interfering RNA (siRNA; Dharmacon) using Lipofectamine 2000 (Invitrogen) alone or in combination with 2.5% or 1.0% O2.

Statistical Analysis
All of the values were expressed as means±SEMs, and P<0.05 was considered significant.

Results
AHR KO Mice Are Hypotensive at Low Altitude and Hypertensive at Modest Altitude
To investigate whether blood pressure of AHR KO mice was affected by altitude, blood pressure of AHR wild-type (WT) and KO male mice was measured by radiotelemetry at Michigan State University (225 m) and the University of New Mexico (1632 m). All of the mice were born and raised at the University of New Mexico, and those studied at low altitude were shipped to Michigan State University at 3 months of age. Mean arterial pressure (MAP) of AHR WT mice did not differ with altitude. However, compared with age-matched AHR WT controls, MAP of AHR KO mice was significantly higher at a modest altitude of 1632 m but was significantly lower at 225 m (Figure 1). In addition, changes in MAP with altitude were paralleled by similar changes in diastolic and systolic blood pressure, but there were no differences in heart rate between genotypes or locations (data not shown).

Plasma ET-1 in AHR KO Mice Is Increased at Low Altitude and Is Increased Further at Modest Altitude
Because previous research demonstrated that hypertension in AHR KO mice at modest altitude is associated with elevated ET-1 and mediated by the endothelin A receptor,2 we measured plasma ET-1 from AHR WT and KO mice at 225 and 1632 m. Plasma ET-1 was significantly increased in AHR KO mice at both altitudes, compared with AHR WT mice; however, the levels of ET-1 were significantly higher in AHR KO mice at 1632 m compared with AHR KO mice at 225 m (Figure 2A).

Pulmonary Prepro-ET-1 mRNA in AHR KO Mice Is Decreased at Modest Altitude and Is Decreased Further at Low Altitude
To determine whether the increased circulating ET-1 was associated with increased prepro-ET-1 mRNA expression, we measured pulmonary prepro-ET-1 mRNA from AHR WT and KO mice at 225 and 1632 m. In contrast to plasma ET-1 levels, prepro-ET-1 mRNA expression was significantly reduced in AHR KO mice at modest and low altitude compared with AHR WT mice. However, as was observed for plasma ET-1, the levels of prepro-ET-1 mRNA ET-1 were significantly higher in AHR KO mice at 1632 m compared with AHR KO mice at 225 m (Figure 2B).

MAP and ET-1 Expression in AHR KO Mice Vary With PIO2
To establish whether differences in PIO2 contributed to the changes in MAP and ET-1 levels in AHR KO mice at different altitudes, baseline MAP in 4-month-old AHR WT and KO mice was measured by radiotelemetry for 6 days at
modest altitude (1632 m; PIO₂: 122 mm Hg), and then mice were exposed to simulated sea level PIO₂ (150 mm Hg) for 11 days. After radiotelemetry, plasma ET-1 and pulmonary prepro-ET-1 mRNA were quantified. MAP of AHR WT mice did not vary with PIO₂; however, MAP of AHR KO mice residing at 1632 m steadily declined when mice were exposed to simulated sea level PIO₂ (Figure 3A). After the 11-day exposure, MAP of AHR KO mice was significantly reduced compared with pre-exposure values and was not significantly different from AHR WT mice (Figure 3B). Furthermore, MAP of AHR KO mice exposed to simulated sea level PIO₂ was not different from MAP of AHR KO mice at low altitude (225 m; PIO₂: 149 mm Hg). Changes in MAP with PIO₂ were paralleled by similar changes in diastolic and systolic blood pressure, whereas there were no differences in heart rates between genotypes or with PIO₂ (data not shown).

In addition, plasma ET-1 was significantly elevated in AHR KO mice residing at 1632 m, declined after exposure to simulated sea level PIO₂ for 11 days, and was not significantly different from AHR KO mice at low altitude (225 m; PIO₂: 149 mm Hg; Figure 4A). Although pulmonary prepro-ET-1 mRNA expression in AHR KO mice residing at 1632 m also declined after exposure to simulated sea level PIO₂ for 11 days, levels remained significantly lower than AHR WT mice under similar exposure conditions and significantly higher than AHR KO mice at 225 m (Figure 4B).

**Arterial Blood Gases and pH Vary With PIO₂ and AHR Genotype**

Because hypoxemia is a potent stimulus of ET-1, we measured arterial blood gases and pH in AHR WT and KO mice residing at 1632 m and after exposure to simulated sea level PIO₂ for 24 hours (acute) or 11 days (chronic; Table 1). AHR KO mice residing at 1632 m were significantly hypoxic, hypercapnic, and acidotic compared with WT mice. When AHR KO mice were exposed acutely to simulated sea level PIO₂, both arterial pO₂ and pCO₂ increased, although the mice remained significantly hypoxic. Notably, however, when AHR KO mice were exposed chronically to simulated sea level PIO₂, arterial blood gases and pH were completely normalized. Finally, hematocrit and hemoglobin were lower in AHR KO mice compared with AHR WT mice, but this difference was only significant after chronic exposure to simulated sea level PIO₂ (Table 2).

**Inactivation of AHR in Endothelial Cells Reduces Basal Prepro-ET-1 mRNA Expression but Fails to Alter Hypoxia-Inducible Expression or Secretion**

To determine whether AHR suppressed hypoxic induction of ET-1 expression, we transfected human microvascular endothelial cells isolated from the lung with control or AHR siRNA and measured prepro-ET-1 mRNA expression and ET-1 secre-
ET-1 secretion (data not shown). Similar results were obtained for prepro-ET-1 expression (Figure 5B) and had no effect on hypoxia-induced pulmonary hypertension under normoxic conditions (Figure 5A) but failed to alter hypoxia-induced pulmonary hypertension. Successful AHR inactivation was confirmed using methods demonstrated in Figure 4. Plasma ET-1 and pulmonary prepro-ET-1 mRNA expression above basal levels were observed in WT mice. Hypoxia-induced pulmonary hypertension is associated with increased circulating and pulmonary ET-1 expression, and disease pathogenesis is ET-1-dependent. In AHR KO mice, hypoxia induces circulating ET-1 and pulmonary prepro-ET-1 mRNA, although the pulmonary mRNA expression remains significantly lower than AHR WT mice, and AHR KO mice develop systemic hypertension in the absence of symptoms of pulmonary hypertension. AHR KO mice do not exhibit polycythemia and, in fact, show a slight decrease in hematocrit. In addition, AHR KO mice do not exhibit an increase in right ventricle weight (Table S1), characteristic of increased pulmonary arterial pressure, but rather exhibit an increase in left ventricle weight, characteristic of increased systemic arterial pressure. Although most studies report pulmonary hypertension after chronic exposure to severe hypoxia (PIO2 of 72 mm Hg), ≥3 genetic models develop pulmonary hypertension after chronic exposure to modest hypoxia (PIO2: 122 mm Hg). Thus, it is possible that AHR KO mice develop hypoxia-induced systemic hypertension, rather than pulmonary hypertension, because of the failure to induce pulmonary prepro-ET-1 mRNA expression above basal levels observed in WT mice.

There is a limited number of reports of systemic hypertension after chronic exposure to severe hypoxia (PIO2 of 72 to 85 mm Hg). These increases in systemic blood pressure are associated with increases in ET-1 and pulmonary arterial pressure, and neither a nonselective endothelin A/B receptor antagonist nor a selective endothelin A receptor antagonist reduces the systemic hypertension. Thus, the mechanism underlying the development of systemic hypertension in AHR KO mice at modest altitude appears to differ from that associated with other models of altitude-induced systemic hypertension. Nonetheless, the PIO2-dependent changes in blood pressure in the AHR KO mice provide a potential explanation for different blood pressure values reported by explanation after exposure of cells to 21.0% 2.5%, or 1.0% O2. Successful AHR inactivation was confirmed using methods reported elsewhere. Inactivation of AHR in human microvascular endothelial cells isolated from lung significantly reduced prepro-ET-1 mRNA expression under normoxic conditions (Figure 5A) but failed to alter hypoxia-induced expression (Figure 5B) and had no effect on hypoxia-induced ET-1 secretion (data not shown). Similar results were obtained in studies conducted with human umbilical vein endothelial cells (data not shown).

**Table 1. Arterial Blood Gases, pH, and Total CO2 of AHR WT and KO Mice Residing at 1632 m and After Acute (24-Hour) or Chronic (11-Day) Exposure to Simulated Sea Level PIO2**

<table>
<thead>
<tr>
<th>Arterial Blood Parameter</th>
<th>1632 m</th>
<th>Acute Simulated Sea Level PIO2</th>
<th>Chronic Simulated Sea Level PIO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AHR WT (N=6)</td>
<td>AHR KO (N=5)</td>
<td>AHR WT (N=6)</td>
</tr>
<tr>
<td>PO2, mm Hg</td>
<td>71 ± 3.1</td>
<td>54 ± 4.1*</td>
<td>86 ± 4.2</td>
</tr>
<tr>
<td>PCO2, mm Hg</td>
<td>27.3 ± 0.8</td>
<td>31.3 ± 1.7*</td>
<td>32.2 ± 2.2</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td>94.0 ± 1.1</td>
<td>81.6 ± 3.2*</td>
<td>96.2 ± 0.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.03</td>
<td>7.26 ± 0.03*</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>TCO2, mmol/L</td>
<td>18.2 ± 0.8</td>
<td>15.2 ± 1.8*</td>
<td>19.5 ± 0.7</td>
</tr>
</tbody>
</table>

*TcO2 indicates total CO2.

*P < 0.05 vs AHR WT at 1632 m.
†P < 0.05 vs AHR WT at acute simulated sea level PIO2.
‡P < 0.05 vs AHR KO at 1632 m.

Discussion

These results establish the AHR KO mouse as a genetic model where systemic blood pressure varies with a small change in inspired pO2, and systemic hypertension develops at a modest altitude. This response is unique, because both humans and animal models typically develop pulmonary hypertension, right ventricular hypertrophy, and polycythemia with increases in altitude and decreases in PIO2. Hypoxia-induced pulmonary hypertension is associated with increased circulating and pulmonary ET-1 expression,13–15 and disease pathogenesis is ET-1-dependent. In AHR KO mice, hypoxia induces circulating ET-1 and pulmonary prepro-ET-1 mRNA, although the pulmonary mRNA expression remains significantly lower than AHR WT mice, and AHR KO mice develop systemic hypertension in the absence of symptoms of pulmonary hypertension. AHR KO mice do not exhibit polycythemia and, in fact, show a slight decrease in hematocrit. In addition, AHR KO mice do not exhibit an increase in right ventricle weight (Table S1), characteristic of increased pulmonary arterial pressure, but rather exhibit an increase in left ventricle weight, characteristic of increased systemic arterial pressure. Although most studies report pulmonary hypertension after chronic exposure to severe hypoxia (PIO2 of 72 mm Hg), ≥3 genetic models develop pulmonary hypertension after chronic exposure to modest hypoxia (PIO2: 122 mm Hg). Thus, it is possible that AHR KO mice develop hypoxia-induced systemic hypertension, rather than pulmonary hypertension, because of the failure to induce pulmonary prepro-ET-1 mRNA expression above basal levels observed in WT mice.

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others studying this KO model. Furthermore, our studies, as well as those of others, provide evidence that the AHR signaling pathway may play a role in the pathogenesis of systemic hypertension. Both the AHR and AHR-regulated gene cytochrome P4501A1 map to quantitative trait loci associated with hypertension, and human hypertension is associated with polymorphisms in both AHR and cytochrome P4501A1, as well as chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Thus, the mechanism by which AHR signaling regulates blood pressure and impacts the etiology and pathogenesis of hypertension requires further investigation.

The elevated levels of plasma ET-1 in AHR KO mice at low altitude suggest that there is increased production and/or decreased clearance of ET-1 under normoxic conditions. If production of ET-1 is increased, the source remains to be identified; however, our data would suggest that the lung is not the source, because pulmonary prepro-ET-1 mRNA is significantly reduced at low altitude, and ET-1 has been shown to be regulated primarily at the transcriptional level. Alternatively, if clearance is reduced, one possible explanation is that it results from a significantly smaller liver (Table S1). Adult AHR KO mice exhibit a liver that is 25% to 30% smaller than WT mice as a result of a persistent ductus venosus, which shunts blood away from the liver. Although the lung is the primary site for ET-1 clearance, studies have shown that the liver is an important site for ET-1 clearance, and circulating ET-1 increases significantly when hepatic function normally in AHR WT mice at modest altitude but may not in AHR KO mice. Thus, the hypoxemia exhibited by these mice. Hypoxemia is a potent inducer of ET-1 in humans and experimental animals, and arterial pO2 <65 mm Hg is associated with significant increases in plasma ET-1 in humans. Furthermore, the degree of hypoxemia correlates with induction of pulmonary prepro-ET-1 mRNA expression in the AHR KO mice, although the levels remain significantly less than WT mice. Thus, the hypoxemia exhibited by AHR KO mice at modest altitude could be responsible for the increase observed in plasma ET-1 above the already elevated basal levels.

Our data do not support the hypothesis that AHR KO mice are generally more sensitive to hypoxic induction of gene expression. Although AHR KO mice were significantly hypoxicemic, hematocrit was not increased as might be expected if the mice were more sensitive to hypoxic induction of erythropoietin. Furthermore, AHR deficiency in pulmonary endothelial cells does not alter the sensitivity to hypoxic induction of prepro-ET-1 mRNA. These observations are in contrast to the enhanced response of AHR KO mice to ischemia-induced expression of vascular endothelial growth factor and neovascularogenesis, suggesting that the sensitivity of AHR KO mice to hypoxic induction of gene expression may be tissue and/or gene dependent.

The presence of mild hypoxemia and hypercapnia in AHR KO mice at modest altitude was unexpected, and the mechanism underlying this observation is not known. Decreases in arterial pO2 are detected rapidly by the carotid body, which stimulates a persistent increase in ventilation, increasing arterial pO2 and decreasing arterial pCO2. Based on the arterial blood gas data, this ventilatory response appears to function normally in AHR WT mice at modest altitude but may not in AHR KO mice. Alternatively, the hypoxemia in AHR KO mice could result from alterations in lung morphology that reduce diffusion capacity or from a ventilation-

### Table 2. Hematocrit and Hemoglobin of AHR WT and KO Mice Residing at 1632 m and After Chronic (11-Day) Exposure to Simulated Sea Level PIO2

<table>
<thead>
<tr>
<th>Venous Blood Parameter</th>
<th>1632 m</th>
<th>Chronic Simulated Sea Level PIO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AHR WT (N=9)</td>
<td>AHR KO (N=8)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.49±0.01</td>
<td>0.45±0.01</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>16.5±0.4</td>
<td>15.4±0.4</td>
</tr>
</tbody>
</table>

*P<0.005 vs AHR WT at chronic simulated sea level PIO2.
perfusion mismatch. Future studies will need to investigate the contributions of these factors to the hypoxemia.

Finally, the reason for the acidosisis observed in AHR KO mice at modest altitude also remains to be determined but probably is not a result of hypercapnia. Although arterial pH decreases as arterial pCO2 increases, the degree of acidosisis in AHR KO mice is more severe than would be predicted by the mild degree of hypercapnia. Given that chronic exposure to simulated sea level PIO2 normalizes the acidosisis, it is possible that hypoxemia-induced lactic acidosisis may be the underlying cause, although this needs to be investigated further.

In conclusion, our studies establish the AHR KO mouse as a genetic model in which modest decreases in PIO2 lead to significant hypoxemia, increased ET-1, and systemic hypertension in the absence of symptoms of pulmonary hypertension.

**Perspectives**

Although studies show that people living at high altitudes tend to have lower systemic blood pressure, these differences are only observed after years of residence. In contrast, short visits to modest or high altitudes induce significant increases in systemic blood pressure, and preexisting genetic factors may predispose low altitude residents to altitude-induced hypertension. Our data suggest that the Ahr may represent a candidate gene for altitude-induced hypertension. Further study of AHR KO mice may reveal novel pathways in the regulation of systemic blood pressure by hypoxia and identify physiological mechanisms that contribute to altitude-induced systemic hypertension.

**Acknowledgments**

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**Disclosures**

None.

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Running title: Altitude-induced Hypertension in AHR Null Mice

On-line supplement

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Online Data Supplement

Expanded Materials and Methods

Chemicals. Lipofectamine 2000 and Opti-MEM I reduced-serum media were purchased from Invitrogen (Carlsbad, CA).

Animals. The aryl hydrocarbon receptor knockout (AHR KO) mice were obtained from Dr. Frank Gonzalez (National Cancer Institute) and backcrossed 11 generations to C57Bl/6N (Harlan). Mice were fed ad libitum and maintained on a 12/12 light/dark cycle. C57Bl/6N AHR wildtype (WT) mice were purchased from Harlan at 6-8 wks of age. At the time of euthanasia, mice were deeply anesthetized with ketamine/xylazine (80 mg/kg and 4 mg/kg, respectively) and blood was collected into a heparinized syringe by cardiac puncture. Lungs were removed and snap frozen. Blood was centrifuged for 10 min at 950 x g to separate plasma, which was stored at -20° until analyzed.

Blood Pressure Analysis. To implant the radiotelemetry transmitters (PA-C20), mice were injected with buphrenorphine (0.1 mg/kg, subq) 30 min prior to ketamine (91 mg/kg, ip) and acepromazine (0.9 mg/kg, ip) to induce anesthesia. The left carotid artery was exposed, and after separating jugular vein and nerves, the artery was securely ligated with sutures and catheter inserted to 1.0 cm from the carotid artery bifurcation. A tunnel was created under the skin and body of the transmitter was placed subcutaneously in the right abdominal area. The mouse was kept warm and observed until completely conscious. Beginning seven days after surgery,
baseline blood pressure values, including mean, systolic, and diastolic; and heart rate were recorded for 10 s every 15 min for 24 hr/d for a total of 6 d (Data Sciences International, Minneapolis, MN). At UNM, after recording baseline blood pressure values the mice were placed into a Plexiglass chamber where the PIO2 was maintained at 150 mm Hg for 11 d by infusion of oxygen and blood pressure values continued to be recorded.

**Arterial Blood Gas Analysis.** Microrenathane (Braintree Scientific, Braintree, MA) was stretched to < 400 µm using a heat gun and sesame oil, and a suture anchor point was added using silastic adhesive. Mice were injected with buphrenorphine (0.1 mg/kg, subq) and anesthetized with isoflurane. The catheter was filled with a heparin/glycerol (50 U/ml), inserted into the left carotid artery, and tunneled subcutaneously where it exited loosely between the scapula. Catheters were flushed twice per day with heparinized saline (50 U/ml). In the first group of mice residing at 1632 m, an arterial blood sample was drawn from conscious animals two days post surgery and PO2, PCO2, oxygen saturation, pH, and total CO2 were measured using an i-STAT 1 analyzer (Abbott Point of Care Inc., East Windsor, NJ). These same mice were then placed into a Plexiglass chamber where PIO2 was maintained at simulate sea level (150 mm Hg) and after 24 hr a second arterial blood sample was drawn without removing the animal from the chamber, and blood gases analyzed. In the second group of mice residing at 1632 m, mice were placed into the oxygen chamber and exposed to simulated sea level PIO2 for 11 d. On day 9 the mice were removed from the chamber to implant an arterial catheter (~45 min) and then returned to the chamber for an additional two days. After a total of 11 d at simulated sea level PIO2, an arterial blood sample was drawn and analyzed. In a third group of mice, a blood sample was collected from the mandibular vein and then the mice were placed into
the oxygen chamber and exposed to simulated sea level PIO₂ for 11 d when a second blood sample was collected. These samples were analyzed for hematocrit and hemoglobin with the i-STAT analyzer.

**Analysis of ET-1 Expression.** Plasma ET-1 was concentrated using C2 Amprep minicolumns (Amersham Pharmacia, Piscataway, NJ) and assayed by RIA (Amersham Pharmacia), while ET-1 from cell culture media was analyzed by ELISA (R&D Systems, Minneapolis, MN). For proET-1 mRNA analysis, total RNA was isolated from the lung (Trizol, Sigma-Aldrich, St. Louis, MO) and endothelial cells (RNeasy®, QIAGEN, Valencia, CA), cDNA was synthesized (iScript Select, Bio-Rad, Hercules, CA) with random primers at 42°C for 30 min, and quantitative real-time PCR analysis conducted with SYBR green PCR Master mix (Bio-Rad), a Bio-Rad iCycler optical system, and phosphoglycerate kinase (PGK1) mRNA as the internal normalization control for lung tissue and 18s rRNA for the cultured endothelial cells.

Primers were designed using Beacon Designer software and PCR reaction efficiency was determined (efficiency = e^{ln10/s} - 1, s=standard curve slope) and optimized >99%. Amplification of a single, appropriately sized band will be confirmed by gel electrophoresis and melt-curve analyses. Amplification of target and PGK cDNA were run side-by-side in triplicate. Threshold crossing value (CT) was calculated using well factor background subtraction, and gene expression normalized to PGK1 or 18s for each sample. The mouse PCR primer sequences were: ET-1 sense: 5’ AAGACCATCTGTGTGGCTTCTAC 3’; ET-1 antisense: 5’ CAGCCTTTCTTTGTGTGGAT 3’; PGK1 sense: 5’ CAAGCTACTGTGGCCTCTGGT 3’; and PGK1 antisense: 5’ CGGCATATTCTTTGCTGCTCTC 3’, while the human PCR primer
sequences were: ET-1 sense: 5’ CATTGGTGACAGACCTTCGGG 3’; ET-1 antisense: 5’ GATGCTCCTGCTCTGATCCCA 3’; 18s sense: 5’ CGGAGGTTCGAAGACGATCAGATA 3’; 18s antisense: 5’ TTGGTTTCCCGGAAGCTGCC 3’.

**Cell Culture and siRNA Transfection.** HUVEC and HMVEC-L were purchased from Lonza (Walkersville, MD) and cultured in microvascular endothelial cell media-2 (EGM-2MV, Lonza), which contained 5% fetal bovine serum, 0.1% antibiotics (GA-1000) and other supplements, at 37°C in 21% O2 and 5% CO2. For transfections, cells were plated at 2 x 10^5 per well (~ 80% confluence) in EGM-2MV without antibiotics and the next day Lipofectamine 2000, mixed with Opti-MEM I reduced-serum media and either control siRNA or AHR ON-TARGETplus SMARTpool siRNA (Dharmacon, Chicago, IL), was added to cells for 48 hrs. When the cells were exposed to siRNA and hypoxia, hypoxia exposures were performed during the last 18 hrs of the 48-hr siRNA treatment period. For hypoxia, cells were exposed to 2.5 or 1% O₂ using nitrogen infusion in a Napco 7001H incubator (Fisher Scientific, Pittsburgh, PA).

**Statistical Analysis.** Altitude- and genotype-related changes on MAP and ET-1 were analyzed by two-way analysis of variance (ANOVA) with post hoc Holm-Sidak comparisons. Inspired PIO2-, time-, and genotype-related changes on MAP were analyzed by repeated measures, two-way ANOVA with post hoc Holm-Sidak comparisons. Arterial blood gases were compared between genotypes by an unpaired Student’s t-test and within a genotype by a paired and unpaired Student’s t-test when appropriate. Oxygen- and siRNA-related changes on AHR and proproET-1 mRNA expression, and ET-1 secretion in endothelial cells were analyzed by two-way ANOVA with post hoc Holm-Sidak comparisons. \( P<0.05 \) was considered statistically
significant in all cases.
Table S1. Body, heart, and liver weights (g) of AHR WT and KO mice at 4 mo of age.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>BW</th>
<th>HW</th>
<th>HW/BW %</th>
<th>LV+S</th>
<th>LV+S/BW %</th>
<th>RV</th>
<th>RV/BW %</th>
<th>RV/LV+S</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>8</td>
<td>27.8±0.7</td>
<td>0.117±0.003</td>
<td>0.42±0.02</td>
<td>0.088±0.002</td>
<td>0.32±0.01</td>
<td>0.029±0.001</td>
<td>0.10±0.01</td>
<td>0.33±0.02</td>
<td>0.052±0.0007</td>
</tr>
<tr>
<td>KO</td>
<td>6</td>
<td>30.1±1.1</td>
<td>0.143±0.006*</td>
<td>0.48±0.02</td>
<td>0.112±0.004*</td>
<td>0.37±0.02*</td>
<td>0.031±0.003</td>
<td>0.10±0.01</td>
<td>0.28±0.02</td>
<td>0.036±0.0003*</td>
</tr>
</tbody>
</table>

Abbreviations: BW, body weight; HW, heart weight; LV+S, left ventricle plus septum; RV, right ventricle

*P<0.05 compared to AHR WT