Endothelin B receptor deficiency potentiates ET-1 and hypoxic pulmonary vasoconstriction

D. DUNBAR IVY,1,2 IVAN F. MCGRUTY,3 MASASHI YANAGISAWA,4 CHERYL E. GARIPEPY,4 TIMOTHY D. LE CRAS,1,5 SARAH A. GEBB,1,5 KENNETH G. MORRIS,3 RICHARD C. WISEMAN,4 AND STEVEN H. ABMAN1,3
1Pediatric Heart Lung Center and Sections of2Pediatric Cardiology, 5Pediatric Pulmonary Medicine, and3Cardiovascular Pulmonary Research Laboratory, University of Colorado School of Medicine and The Children's Hospital, Denver, Colorado 80218; and 4Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75390

Received 11 November 2000; accepted in final form 21 November 2000

Ivy, D. Dunbar, Ivan F. McMurtry, Masashi Yanagisawa, Cheryl E. Gariepy, Timothy D. Le Cras, Sarah A. Gebb, Kenneth G. Morris, Richard C. Wiseman, and Steven H. Abman. Endothelin B receptor deficiency potentiates ET-1 and hypoxic pulmonary vasoconstriction. Am J Physiol Lung Cell Mol Physiol 280: L1040–L1048, 2001.—Endothelin (ET)-1 contributes to the regulation of pulmonary vascular tone by stimulation of the ETA and ETB receptors. Although activation of the ETA receptor causes vasoconstriction, stimulation of the ETB receptors can elicit either vasodilation or vasoconstriction. To examine the physiological role of the ETB receptor in the pulmonary circulation, we studied a genetic rat model of ETB receptor deficiency (transgenic(sl/sl)). We hypothesized that deficiency of the ETB receptor would predispose the transgenic(sl/sl) rat lung circulation to enhanced pulmonary vasoconstriction. We found that the lungs of transgenic(sl/sl) rats are ETB deficient because they lack ETB mRNA in the pulmonary vasculature, have minimal ETB receptors as determined with an ET-1 radioligand binding assay, and lack ET-1-mediated pulmonary vasodilation. The transgenic(sl/sl) rats have higher basal pulmonary arterial pressure and vasopressor responses to brief hypoxia or ET-1 infusion. Plasma ET-1 levels are elevated and endothelial nitric oxide synthase protein content and nitric oxide production are diminished in the transgenic(sl/sl) rat lung. These findings suggest that the ETB receptor plays a major physiological role in modulating resting pulmonary vascular tone and reactivity to acute hypoxia. We speculate that impaired ETB receptor activity can contribute to the pathogenesis of pulmonary hypertension.

Contrast, ETB receptors are present on both endothelial and smooth muscle cells in the rat pulmonary circulation (6, 13, 14). Stimulation of endothelial ETB receptors causes vasodilation through the release of nitric oxide (NO) and prostaglandins and also functions to remove ET-1 from the circulation (9, 15, 30). Stimulation of ETB receptors on smooth muscle causes vasoconstriction in the rat lung (30). Consequently, the physiological role of ET-1 in the regulation of vascular tone in the rat lung is complex, and the exact role of the ETB receptor in the regulation of pulmonary tone remains unclear.

The spotting lethal rat is a naturally occurring rat strain that carries a 301-bp deletion in the ETB gene, rendering the gene nonfunctional. This autosomal recessive mutation leads to coat color spotting and intestinal aganglionosis, with resultant intestinal obstruction and death shortly after birth (10). Rescue of the spotting lethal rat by transgenic expression of the ETB gene prevents intestinal aganglionosis and death (11). Transgenic rats harboring a wild-type rat ETB receptor cDNA, the expression of which is driven by the human dopamine-beta-hydroxylase (DBH) promoter, were generated by Dr. M. Yanagisawa. The 5.8-kb fragment of the human DBH promoter drives ETB expression in the neural crest-derived enteric nervous system precursors as they colonize the developing gut. Rats carrying the transgene were crossed with spotting lethal rats to produce rats that express ETB only under the transcriptional control of the DBH promoter (DBH transgene(+/+), ETB receptor(sl/sl)). Yanagisawa and colleagues have called the DBH transgene(+/+), ETB receptor(sl/sl) rat ETB deficient. For the purposes of this paper, the DBH transgene(+/+), ETB receptor(sl/sl) rat will be abbreviated as transgenic(sl/sl). The ETB-deficient rats exhibit normal enteric nervous system development and live into adulthood (11). Although the ETB-deficient rats lack expression of ETB.

Address for reprint requests and other correspondence: D. D. Ivy, Dept. of Cardiology, Box B100, The Children’s Hospital, 1056 E. 19th Ave., Denver, CO 80218 (E-mail: dunbar.ivy@uchsc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1040-0605/01 $5.00 Copyright © 2001 the American Physiological Society

http://www.ajplung.org
driven by the endogenous promoter, they express ET\textsubscript{B} in adrenergic tissues such as the adrenal medulla and sympathetic ganglion. Recent data (11) suggest that the transgenic(sl/sl) rat develops salt-dependent systemic hypertension and lacks ET\textsubscript{B} receptor-mediated systemic vasodilation. Similarly, deficiency of the ET\textsubscript{B} receptor in the systemic circulation of mice leads to systemic hypertension (28). However, little is known about the effects of ET\textsubscript{B} receptor deficiency in the pulmonary circulation.

Three specific questions were addressed in this study. 1) Is the pulmonary circulation of the transgenic(sl/sl) rat ET\textsubscript{B} receptor deficient? 2) Do transgenic(sl/sl) rats have increased basal pulmonary arterial pressure (PAP) and total pulmonary resistance (TPR)? 3) Do rats deficient in the ET\textsubscript{B} receptor have an abnormal pulmonary vasopressor response to acute hypoxia and ET-1?

MATERIALS AND METHODS

**Animals.** A colony of four groups of rats was studied: wild-type control [DBH transgene(−/−), ET\textsubscript{B} receptor(+/+)], transgenic control [DBH transgene(+/+), ET\textsubscript{B} receptor(+/+)], heterozygous [DBH transgene(+/+), ET\textsubscript{B} receptor(sl/sl)], and transgenic(sl/sl) [DBH transgene(+/+), ET\textsubscript{B} receptor(sl/sl)]. Ninety animals were used in these protocols. The colony was established with founder animals provided by Dr. M. Yanagisawa (11). The animals were exposed to a 12:12-h light-dark cycle and fed standard rodent chow and water. The genotype of each animal was confirmed with PCR of genomic DNA with standard techniques as previously described (11).

**In situ hybridization for the ET\textsubscript{B} receptor.** Tissue for in situ hybridization [wild type, n = 3 rats; transgenic(+/+), n = 3 rats; transgenic(sl/sl), n = 3 rats] was fixed overnight in freshly prepared 4% paraformaldehyde in RNase-free phosphate-buffered saline (PBS). Fixed tissue was dehydrated and paraffin embedded, and 4- to 6-μm sections were cut and mounted on Super Frost Plus (Fisher) microscope slides. In situ hybridization was performed as described by Deterding and Shannon (4), with the exception that the cRNA probes were labeled with [\textsuperscript{35}P]UTP. Both sense and antisense ET\textsubscript{B} receptor riboprobes were generated with the appropriate linearized templates of a 770-bp ET\textsubscript{B} fragment (Abbott Laboratories). The ET\textsubscript{B} receptor transcripts were hydrolyzed to yield fragments of 300–400 bp. After hybridization and high-stringency washes, the slides were dipped in NTB-2 nuclear track emulsion (Eastman Kodak, Rochester, NY) and developed 30 days later. All sections were hybridized at the same time, and the exposure times of the autoradiographs were identical.

**Radioligand binding assay.** Membrane fractions were prepared from frozen rat lung tissues of wild-type (n = 4) and transgenic(sl/sl) (n = 4) rats. Binding assays were carried out in triplicate with 10\textsuperscript{−11} M \textsuperscript{125}I-ET-1 as a tracer and 90 μg membrane protein/reaction. Nonspecific binding was defined in the presence of 10\textsuperscript{−6} M unlabeled ET-1 and was ~5% of total binding in the wild-type membranes. ET\textsubscript{B} binding was determined in the presence of the selective ET\textsubscript{A} receptor antagonist FR-139317 (10\textsuperscript{−6} M) (10, 16).

**Hemodynamic studies in conscious catheterized rats.** Hemodynamic measurements were performed in chronically instrumented rats as previously described (24). A total of 38 animals were used in the study of right ventricular hypertrophy and histology in conscious catheterized rats [wild type, n = 11; transgenic(+/+), n = 11; transgenic(sl/+), n = 7; transgenic(sl/sl), n = 9]. Male and female rats at 3 mo of age were anesthetized with ketamine-xylazine (100 mg/kg and 15 mg/kg, respectively) for the placement of catheters in the right carotid artery, main pulmonary artery (PA), and jugular vein. Blood pressure tracings identified the intravascular location of the catheter tips. The catheters were filled with heparinized saline (10 U/ml of heparin in 0.9% saline), sealed, tunneled subdermally to the back of the neck, and then exteriorized and enclosed in a small plastic container. After 24 h of recovery, the conscious rats were placed in a clear plastic chamber during hemodynamic measurements and pulmonary and systemic blood pressure determinations. Cardiac output (CO) was measured by the standard dye-dilution technique and calculated by computer. Hemodynamic measurements were performed while all the rats were breathing room air and subsequently while breathing hypoxic gas (inspired O\textsubscript{2} fraction = 0.10 for 10 min). TPR (in mmHg-l\textsuperscript{−1}-min\textsuperscript{−1}) was calculated as mean PAP/CO. Arterial blood samples were drawn into heparinized syringes through the carotid arterial catheter. Arterial PO\textsubscript{2}, P\textsubscript{O\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}}, and pH were measured with a clinical blood gas analyzer (Radiometer, Copenhagen, Denmark). Hematocrit was measured with a capillary tube and standard techniques.

**Right ventricular hypertrophy.** Immediately after death, the heart was resected, and the atria were removed to the plane of the atrioventricular valves. The right ventricle (RV) free wall was then dissected free of the left ventricle (LV) and septum (S). The RV and LV+S were weighed, and the RV-to-LV+S ratio was calculated.

**Histology and morphometrics.** Rat lungs were fixed for histology by tracheal instillation of 10% buffered formalin under constant pressure (10 cmH\textsubscript{2}O). The trachea was ligated after sustained inflation, and the lungs were excised and immersed in formalin overnight. Formalin-fixed lung tissue was cut into 4- to 5-mm-thick sections, placed in 10% buffered formalin, and embedded in paraffin. Paraffin sections (5 μm thick) were mounted and stained. A representative hematoxylin- and eosin-stained section was then coded and evaluated in a blinded manner for measurement of wall thickness of PAs <100 μm located at the level of the terminal bronchiol, respiratory bronchiole, or alveolar duct. Four animals per group were examined, and 10 measurements were obtained from each study animal. Measurements of external diameter and medial wall thickness were obtained with the Zeiss Interactive Digital Analyzer System (Carl Zeiss, Thornwood, NY) as previously described (20). The wall thickness of each artery is expressed as a percentage of the external diameter with the formula [(2 × medial wall thickness)/ED] × 100, where ED is external diameter. Measurements were only performed on vessels that were cut transversely.

**Isolated rat lung studies.** Lungs were isolated from 16 rats [wild type, n = 4; transgenic(+/+), n = 4; transgenic(sl/+), n = 4; transgenic(sl/sl), n = 4]. After administration of 30 mg of pentobarbital sodium intraperitoneally and intracardiac injection of 200 IU of heparin, the PA and LV were cannulated, with the heart-lung block remaining intact (30). The lungs were ventilated at an inspiratory pressure of 9 cmH\textsubscript{2}O and end-expiratory pressure of 2.5 cmH\textsubscript{2}O with a humidified mixture of normoxic gas (21% O\textsubscript{2}-5% CO\textsubscript{2}-74% N\textsubscript{2}) for 60 min following by hypoxic gas (0% O\textsubscript{2}-5% CO\textsubscript{2}-95% N\textsubscript{2}) at 60 breaths/min. Perfusion was maintained at 0.04 ml-g body wt\textsuperscript{−1}-min\textsuperscript{−1} with a peristaltic pump (Gilson). Heparinized whole blood obtained from Metofane-anesthetized wild-type donor rats was used for the perfusate. Effluent perfusate was drained from the left ventricular cannula into a reservoir and...
then recirculated (total volume 30 ml). Lung and perfusate temperatures were maintained at 38°C. Perfusate pH was kept between 7.35 and 7.45. The mean PA catheter was connected to a computer-driven pressure transducer and recorder (Biopac, Santa Barbara, CA). The pressure transducers were calibrated with a mercury column manometer. ET-1 (5 nM; Alexis Biochemicals, San Diego, CA) was added to the perfusate, and peak transient vasodilation and sustained vasoconstriction were recorded for 30 min after the addition of ET-1. Samples for cumulative perfusate NOx were drawn before and after ET-1 infusion.

**Measurement of perfusate NOx.** A NO chemiluminescence analyzer (NOA 280, Sievers Research) was used to measure the levels of NOx (NO2, NO3, nitrosothiols, and peroxynitrite) in the lung perfusate. Aliquots of the perfusate (10 μl) were added to 2 ml of 0.1 M vanadium chloride (type III; Aldrich), dissolved in 1 N HCl, and heated to 90°C in the purge vessel of the NO analyzer to reduce all NOx to NO. The liberated NO was driven into the chemiluminescence chamber by bubbling the reaction mixture with argon. Calibration curves for NOx levels were generated daily by measuring the amount of NO produced by a range (10–100 pM) of sodium nitrate solutions (Mallinckrodt) (31).

**Western blot analysis.** Western blot analysis was performed according to previously published techniques by Le Cras et al. (24) with a monoclonal antibody to endothelial NO synthase (eNOS; Transduction Laboratories, Lexington, KY). Briefly, lung tissue [wild type, n = 4 rats; transgenic(+/+), n = 4 rats; transgenic(sl/+), n = 4 rats; transgenic(sl/sl), n = 4 rats] was homogenized on ice in 25 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, 1 mM EGTA, 0.1% (vol/vol) 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 2 mM leupeptin, and 1 mM pepstatin A. The homogenate was centrifuged at 1,500 g at 4°C for 10 min to remove cell debris.
increasing amounts of lung protein from a transgenic (sl/sl) rat showed that there was a linear increase in eNOS protein density. cpm, Counts/min.

**RESULTS**

**In situ hybridization.** In the wild-type and transgenic(+/+) lungs, we found expression of mRNA for the ET B receptor in the endothelium and smooth muscle of PAs as well as of lung parenchyma (Fig. 1). Minimal staining was noted in the proximal airways. A similar signal was seen in the parenchyma and airway epithelium in transgenic(sl/+), ETB receptor in the endothelium and smooth muscle of PAs as well as of lung parenchyma. In contrast, there was no specific ET B mRNA signal in the PAs of the transgenic(sl/sl) animals. The signal was localized to the parenchyma of the transgenic(sl/sl) rat lung.

**Radioligand binding study.** In the lung membrane preparations, there were minimal ET B receptors (FR-139317-resistant binding) in the transgenic(sl/sl) rat lung (Fig. 2). Total ET receptors were decreased 42% in the transgenic(sl/sl) rat compared with those in the wild-type lung. ET B receptor binding accounts for 25% of total ET binding in the wild-type lung.

**Animal and hemodynamic data.** Body weight was not different among the study groups (Table 1). Compared with control animals, RV weight was ~40% greater in the transgenic(sl/) and transgenic(sl/sl) than in the wild-type or transgenic(+/+) animals. LV+S weight was not different between the groups. Right ventricular hypertrophy as determined by the ratio of RV to LV+S weight was greater in the transgenic(sl/) and transgenic(sl/sl) than in either the wild-type or transgenic(+/+) control animals (Table 1). Compared with wild-type or transgenic(+/+) animals, the hematocrit was greater in the transgenic(sl/) and transgenic(sl/sl) animals. Furthermore, the transgenic(sl/sl) animals had a higher hematocrit than the transgenic(sl/) animals (Table 1).

**Table 1.** Body weight, right ventricular hypertrophy, hematocrit, and morphometric changes

<table>
<thead>
<tr>
<th></th>
<th>Wild Type (n = 11)</th>
<th>Trans +/- (n = 11)</th>
<th>Trans sl/+ (n = 7)</th>
<th>Trans sl/sl (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>268±28</td>
<td>292±26</td>
<td>240±28</td>
<td>284±22</td>
</tr>
<tr>
<td>RV, g</td>
<td>0.14±0.02</td>
<td>0.14±0.01</td>
<td>0.20±0.03†</td>
<td>0.21±0.03†</td>
</tr>
<tr>
<td>LV+S, g</td>
<td>0.47±0.05</td>
<td>0.50±0.04</td>
<td>0.48±0.06†</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>RV/LV+S</td>
<td>0.30±0.03</td>
<td>0.28±0.03</td>
<td>0.42±0.06†</td>
<td>0.41±0.04†</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>48±1</td>
<td>46±1</td>
<td>53±2†</td>
<td>57±1†‡</td>
</tr>
<tr>
<td>Wall thickness, %</td>
<td>23±2</td>
<td>23±1</td>
<td>24±2</td>
<td>22±2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Wild type, transgene (+/−); endothelin (ET) B receptor (+/+); Trans +/-, transgene (+/+) ET B receptor (+/+); Trans sl/+ transgene (+/+), ET B receptor (sl/+); Trans sl/sl transgene (+/+), ET B receptor (sl/sl); RV, right ventricle; LV+S, left ventricle plus septum. Significantly different (P < 0.05) from: *wild type; †Trans +/-; ‡Trans sl/+.
There were no apparent differences in the percent wall thickness of small PAs of transgenic(sl/sl) rats compared with that in any other group (Table 1).

During baseline conditions, the mean PAP was higher in the transgenic(sl/+ and transgenic(sl/sl) animals than in the wild-type animals but not in the transgenic(+/+ control animals. Baseline PAP was not different between the wild-type and transgenic(+/+ animals (Fig. 3). CO was not different between the groups (Table 2). In the transgenic(sl/+ and transgenic(sl/sl) animals, TPR was greater than in either the wild-type or transgenic(+/+) animals (Fig. 4). Aortic pressure was increased more in the transgenic(+/+) and transgenic(sl/) animals than in the wild-type animals (Table 2). Furthermore, aortic pressure was higher in the transgenic(sl/) animals than in any other study group (Table 2). Although arterial pH or PCO2 during normoxia was not different, arterial PO2 was slightly decreased in the transgenic(+/+) and transgenic(sl/) animals.

During acute hypoxia, all groups showed a significant increase in PAP (P < 0.05). The increase in mean PAP was greater in the transgenic(sl/) animals (38 ± 2%) than in the wild-type control (22 ± 5%), transgenic(+/+) control (18 ± 5%), and transgenic(sl/) (23 ± 6%) animals (P < 0.05 for all comparisons; Fig. 3). CO was not different among study groups (Table 2). TPR was greater in both the transgenic(sl/) and transgenic(sl/) animals than in either wild-type or transgenic(+/+) animals (Fig. 4). Aortic pressure was not different among the study groups during acute hypoxia (Table 2). During acute hypoxia, arterial blood gas tensions were similar.

**Isolated rat lung studies: perfusate NOx.** To determine whether transgenic(sl/) rats are functionally ETB receptor deficient, we examined the vascular responses to infusions of 5 nM ET-1 in perfused lungs and NO production after ET-1 stimulation as measured by plasma NOx. We found that the baseline perfusion pressure was greater in the transgenic(sl/) and transgenic(sl/) animals than in the wild-type or transgenic(+/+) animals. Acute hypoxia in the isolated lung preparation caused a significant increase in perfusion pressure in all animals; however, the pressure was greater in transgenic(sl/) and transgenic(sl/) animals than in wild-type or transgenic control animals (Fig. 5A). The transgenic(sl/) rats had a different response to exogenous ET-1 compared with other animals (Fig. 5B). In the wild-type, transgenic(+/+) and transgenic(sl/) animals, ET-1 caused a biphasic response, with an initial transient fall in mean PAP and NO production after ET-1 stimulation as measured by plasma NOx.
followed by an increase in mean PAP. In contrast, the transient vasodilator response to ET-1 was completely absent in the transgenic (sl/sl) animals. Instead, ET-1 caused a marked rise in mean PAP, suggesting increased ETA receptor activity.

Perfusate NOx increased after ET-1 stimulation in the wild-type and transgenic control animals (Fig. 6). In contrast, in the perfused lung preparation, there was no increase in cumulative NOx in the transgenic (sl/+ ) and transgenic (sl/sl) animals, suggesting abnormal production of NO.

eNOS protein content. In the transgenic (sl/sl) rats, lung eNOS protein content was 71 ± 8% lower than the control value (Fig. 7). Furthermore, the lung eNOS protein content was lower in the transgenic (sl/sl) animals than in any of the other study groups. Lung eNOS protein content was not different between wild-type and transgenic (+/+ ) animals.

Plasma ET-1 protein analysis. Pulmonary arterial ET-1 levels were eightfold higher in the transgenic (sl/sl) animals compared with each of the study groups (Fig. 8). ET-1 levels in the transgenic (sl/+ ) animals were not different from those in the control animals.

DISCUSSION

To examine the functional role of the ETB receptor in the rat pulmonary circulation, we studied a genetic strain of rats deficient in the ETB receptor. We found
that the lungs of transgenic(s/l/s) rats are ET_B deficient because they lack ET_B mRNA in the pulmonary vasculature, lack ET_B receptors as determined with an ET-1 radioligand binding assay, and do not have ET-1-mediated pulmonary vasodilation. Mean PAP, TPR, and right ventricular hypertrophy were greater in the transgenic(s/l/s) rats than in the wild-type control animals under baseline conditions. Furthermore, the transgenic(s/l/) rats also developed pulmonary hypertension that was similar in magnitude to that in the transgenic(s/l/s) rats. Both the transgenic(s/l/) and transgenic(s/l/s) rats have an exaggerated pulmonary vasopressor response during brief periods of moderate acute hypoxia. The ET_B-deficient rats had greater circulating ET-1 levels and diminished lung eNOS protein content and NO production than control animals. Overall, these findings suggest that deficiency of the ET_B receptor predisposes the pulmonary circulation to the development of pulmonary hypertension that is characterized by diminished NO production and elevated circulating ET-1 levels.

The exact role of the ET_B receptor in the regulation of pulmonary tone in the rat has been controversial. Previous studies (6, 13, 14) have shown that ET_B receptors are present on both endothelial and smooth muscle cells in the rat pulmonary circulation. Endothelial ET_B receptors cause vasodilation through the release of NO and prostaglandins and may clear circulating ET-1(9, 15, 30). ET_B receptors on smooth muscle cause vasoconstriction in the rat lung (30). The ET_B receptor may also contribute to the regulation of systemic vascular tone because ET_B receptor-deficient rats and mice have elevated systemic arterial pressure (11, 28). Recent studies have shown that the endothelial ET_B receptor is upregulated (32) and important in the production of NO (31) in the chronically hypoxic rat lung. The present study highlights the importance of the ET_B receptors in the maintenance of normal pulmonary vascular tone because deficiency of the ET_B receptor predisposes the transgenic(s/l/s) rat to the development of pulmonary hypertension and exaggerated acute hypoxic pulmonary vasoconstriction.

The pulmonary circulation of wild-type and transgenic(+/+) animals is similar despite differences seen in the systemic circulation. In the whole animal and with the isolated perfused lung technique, PAP and PA resistance were similar during conditions of normoxia and hypoxia. ET-1 infusion caused a similar biphasic response in the isolated perfused lung preparation. The rise in NOX during the isolated perfused lung study was similar, and there was no difference in eNOS protein levels between the wild-type and transgenic(+/+) animals. Likewise, plasma ET-1 levels were similar between the two groups. Finally, mRNA for the ET_B receptor was found in the pulmonary vasculature of both groups of animals.

Several factors may contribute to the development of pulmonary hypertension in the ET_B receptor-deficient rat. Diminished NO production or elevated circulating ET-1 levels may predispose this strain to the development of pulmonary hypertension. However, the exact mechanisms of the decrease in lung eNOS protein and increase in circulating ET-1 in the ET_B receptor-deficient rat remain uncertain. The increase in ET-1 levels may be due to decreased clearance of circulating ET-1 by ET_B receptors (9). In bovine pulmonary arterial endothelial cells, ET-1 increases eNOS protein through stimulation of the ET_B receptor as well as eNOS mRNA and nitrate production (37). Thus it is possible that a deficiency of the ET_B receptor may decrease eNOS protein and NO production. Because NO donors decrease ET-1 production in response to hypoxia in vitro (22), diminished NO production may contribute to increased ET-1 production or vice versa. Whether the mild hypoxia at Denver’s altitude contributes to the development of pulmonary hypertension in the trans-

![Graph of eNOS protein content between Wild Type, Trans +/+, Trans sl/+, and Trans sl/sl](image)

* = p < 0.05 vs Trans sl/sl

![Graph of ET-1 levels in the Wild, Trans +/+, Trans sl/+, and Trans sl/sl](image)

* = p < 0.05 vs Wild Type

# = p < 0.05 vs Trans +/+
genic(sl/+)) and transgenic(sl/sl) animals requires further investigation.

That the heterozygous transgenic(sl/+)) rat also develops pulmonary hypertension is interesting. We found that the transgenic(sl/+)) rats have a higher mean PAP, TPR, and right ventricular hypertrophy and a greater hypoxic pressor response than control rats. Although there was no difference between lung eNOS protein content between transgenic(sl/+)) and control rats, there was a strong trend toward an increase in plasma ET-1 levels between transgenic(sl/+)) and control rats (P = 0.08). Furthermore, there was diminished NOx production in the transgenic(sl/+)) rats in response to ET-1 stimulation. That transgenic(sl/+)) rats maintain ET-1-induced pulmonary vasodilation despite a decrease in NOx suggests that other vasodilator systems may remain intact. Studies have suggested that other heterozygous animals may also be predisposed to the development of exaggerated hypoxic pressor responses. A study (8) in eNOS-null mice has shown that heterozygous animals have greater right ventricular hypertrophy than wild-type control animals under conditions of mild hypoxia but not under sea-level conditions. Thus only a partial loss of ETb receptor function may lead to a predisposition to pulmonary hypertension.

Blockade of ETa receptor activity lowers PAP and hypertensive structural changes in other models of pulmonary hypertension (1, 5, 20, 27). However, little is known about the role of the ETb receptor during the development of pulmonary hypertension. Some studies have shown increased lung ETb receptor mRNA expression in models of pulmonary hypertension (25, 32), whereas others have shown decreased lung ETb receptor mRNA expression (18, 35). The variations may be explained by species differences as well as differences in the hypertensive model used. Although combined ETa and ETb receptor blockade prevents and reverses hypoxic pulmonary hypertension in rats (3), the relative contribution of the ETb receptor remains uncertain. The present study indicates that diminished ETb receptor-mediated vasodilation may promote the development of pulmonary hypertension and suggests that ETb receptor deficiency may lead to enhanced ETa receptor activity.

The mechanisms leading to the increased hematocrit in the transgenic(sl/+)) and transgenic(sl/sl) groups are unknown. It is unlikely that the differences in hypoxia may explain the differences in hematocrit because no difference was seen in the arterial PO2 in both the transgenic(sl/+)) and transgenic(sl/sl) groups. A study (7) has shown that erythropoietin production may be related to proximal renal tubular function and that inhibition of sodium reabsorption reduces tubular oxygen consumption. Because the ETb receptor inhibits sodium reabsorption (11), deficiency of the ETb receptor may lead to increased tubular oxygen consumption and increased erythropoietin production. Furthermore, it is unlikely that the elevation in hematocrit alone caused pulmonary hypertension because a recent study (29) has shown that augmented polycythemia does not increase right ventricular hypertrophy and PAP in the normoxic or hypoxic rat.

Circulating ET-1 levels are increased in the human disorder of pulmonary hypertension as well. Elevated immunoreactive ET-1 levels have been found in primary pulmonary hypertension, the Eisenmenger syndrome (2, 12), and children with congenital heart disease and increased pulmonary blood flow (34). Recently, studies (21, 23, 33) in humans with the combined ETA and ETB receptor antagonist bosentan have shown promising initial results in the treatment of congestive heart failure and systemic hypertension. However, the role of ET receptor antagonists in the treatment of pulmonary hypertension remains incompletely understood.

In summary, our studies demonstrate that ETb receptor activity is important in the regulation of pulmonary vascular tone and in the response to acute hypoxia. Both the transgenic(sl/sl) and transgenic(sl/+)) rats developed pulmonary hypertension. Furthermore, both the transgenic(sl/sl) and transgenic(sl/+)) animals developed exaggerated pulmonary vasoconstriction when exposed to acute hypoxia or ET-1 stimulation. We speculate that partial loss of ETb receptor activity may predispose the animals to the development of severe pulmonary hypertension.

We thank Neil Markham and Cheryl Oliver-Pickett for assistance with this study.

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-03823 (to D. D. Ivy) and HL-14985 (to I. F. McMurtry); a March of Dimes Birth Defects Foundation grant (to D. D. Ivy); an American Heart Association, Desert/Mountain Affiliate Grant-in-Aid (to D. D. Ivy); the Leah Bult Pulmonary Hypertension Fund; and the Caitlyn Whitley Cardiology Research Fund.

REFERENCES