



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Endothelial ET(B) Limits Vascular Remodelling and Development of Pulmonary Hypertension during Hypoxia

Citation for published version:

Kelland, NF, Bagnall, AJ, Morecroft, I, Gulliver-Sloan, FH, Dempsie, Y, Nilsen, M, Yanagisawa, M, Maclean, MR, Kotelevtsev, YV & Webb, DJ 2009, 'Endothelial ET(B) Limits Vascular Remodelling and Development of Pulmonary Hypertension during Hypoxia', *Journal of vascular research*, vol. 47, no. 1, pp. 16-22.
<https://doi.org/10.1159/000231717>

Digital Object Identifier (DOI):

[10.1159/000231717](https://doi.org/10.1159/000231717)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Early version, also known as pre-print

Published In:

Journal of vascular research

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Endothelial ET_B Limits Vascular Remodelling and Development of Pulmonary Hypertension during Hypoxia

N.F. Kelland^a A.J. Bagnall^a I. Morecroft^b F.H. Gulliver-Sloan^a Y. Dempsey^b
M. Nilsen^b M. Yanagisawa^c M.R. MacLean^b Y.V. Kotelevtsev^a D.J. Webb^a

^aCentre for Cardiovascular Science, University of Edinburgh, Edinburgh, and ^bIntegrative and Systems Biology, IBSL, University of Glasgow, Glasgow, UK; ^cUT South-Western Medical Centre at Dallas, Dallas, Tex., USA

Key Words

ET_B receptor • Knockout • Endothelin • Pulmonary hypertension

Abstract

Background: We hypothesised that the potential protective effects of endothelial ET_B are important in limiting pulmonary vascular muscularisation, vasoconstriction and the development of pulmonary arterial hypertension in response to hypoxia. **Methods:** EC-specific ET_B knockout mice (EC ET_B^{-/-}) and control mice (ET_B^{+/+}) were subjected to hypobaric hypoxic (10% FiO₂) or normoxic conditions for 14 days before assessment of right ventricular pressure and pulmonary vascular morphology and function. **Results:** During normoxia, no difference in right ventricular pressure was detected between EC ET_B^{-/-} (23.7 ± 1.7 mm Hg) and ET_B^{+/+} mice (20.2 ± 1.5 mm Hg). Hypoxia induced an exaggerated increase in right ventricular pressure in EC ET_B^{-/-} mice (34.4 ± 1.2 mm Hg vs. 24.6 ± 1.4 mm Hg), accompanied by an increase in right ventricular mass. No effect was observed in ET_B^{+/+} mice. Endothelin-1 constricted pulmonary arteries from both groups, although maximum response was similar irrespective of inspired oxygen or genotype. Hypoxia increased the percentage of muscularised vessels in both groups of mice, but the percentage increase was significantly greater in EC

ET_B^{-/-} mice. **Conclusions:** The potential protective effects of endothelial ET_B are important in limiting pulmonary vascular muscularisation and the development of pulmonary arterial hypertension in response to hypoxia.

Copyright © 2009 S. Karger AG, Basel

Introduction

Pulmonary arterial hypertension (PAH) is a progressive condition involving small pulmonary arteries (PAs) characterised by a sustained increase in pulmonary vascular resistance and vascular remodelling leading ultimately to right ventricular failure and premature death [1]. Several lines of evidence implicate endothelin-1 (ET-1) in the aetiology and progression of PAH. First, ET-1 is a potent vasoconstrictor and mitogen [2]. Second, plasma concentration of ET-1 correlates with severity of PAH in animal models [3–5] and patients [6], and third, non-selective and selective endothelin A receptor antagonists improve symptoms and slow the progression of PAH [7, 8]. The lung is an important site of ET-1 production with a concentration of ET-1 five times greater than that seen in other organs [9]. Endothelin B receptor (ET_B) mRNA is expressed abundantly in the lung [10], particularly in distal segments of the pulmonary vascular tree [11], where

it is found in the endothelium and media of pulmonary blood vessels as well as in bronchioles and alveoli.

In contrast, endothelin A receptors (ET_A) are localised to the media of large proximal PAs and veins with relatively little expression in distal arterioles [11–13]. Hypoxia increases the expression of ET-1, ET_A and ET_B throughout the rat lung [10, 12] with histological evidence to suggest a preferential increase in endothelial cell (EC) ET_B expression in distal segments [13]. EC ET_B elicit vasodilatation and anti-mitogenic effects through the release of nitric oxide (NO) and/or prostaglandin I₂ (PGI₂) [14, 15]. Increased ET-1/EC ET_B-mediated vasodilatation has thus been hypothesised to protect against hypoxia-induced vasoconstriction [13], and studies in rats suggest that ET_B deficiency exacerbates monocrotaline- [16] and hypoxia-induced PAH [17]. Pulmonary ET_B also clear ET-1 from the plasma [18], further limiting ET_A-mediated vasoconstrictor and mitogenic effects.

Vascular smooth muscle cell (VSMC) ET_B mediate vasoconstriction in humans [19]. In hypoxic rat models [20] and sheep models of embolism-induced PAH and PAH secondary to aortopulmonary shunting, there is evidence of increased VSMC ET_B-mediated vasoconstriction [21, 22]. Activation of ET_A and ET_B on VSMC by ET-1 also promotes cellular hypertrophy [11] and hence may promote the progression of PAH through muscularisation of small pulmonary arterioles. Thus, with respect to the development of PAH, pulmonary ET_B have the potential to elicit both protective and detrimental effects. Study of the interplay between EC and VSMC ET_B is likely to increase our mechanistic understanding of the role of the endothelin system in the pathogenesis of PAH. We have previously generated EC-specific ET_B knockout (KO) mice that exhibit endothelial dysfunction in the absence of systemic hypertension, with evidence of impaired endogenous NO release and increased plasma ET-1 [23]. Otherwise, they have normal feeding and growth rates, exhibit normal behaviour and are healthy. Here we have used this model to determine whether the potential protective effects of EC ET_B are important in limiting pulmonary vascular muscularisation and the development of PAH during hypoxia.

Methods

Experimental Animals

Mice featuring selective KO of EC ET_B were generated using a Cre-LoxP approach as previously described [23]. Briefly, mice with loxP sites flanking exons 3 and 4 of the ET_B gene ('floxed' mice, ET_B^{fl/fl}) were crossed with mice expressing a Cre recombi-

nase transgene in an EC-restricted pattern (WW/Tie2-Cre) [24] to produce EC-specific ET_B KO mice (EC ET_B^{-/-}). Genotyping to identify the floxed and recombined alleles was performed by Southern blot and by PCR using primers amplifying a sequence spanning the 3' and 5' loxP sites (forward primer: TCA GTT GTA ATG AGA CAC AGA C; reverse primer: AGC CAT AAA GTC ACA GCC ATT C). The Tie2-Cre transgene was detected by PCR as described [24]. Male mice aged 8–12 weeks (weight 25–35 g) were studied and EC ET_B^{-/-} mice compared with ET_B^{fl/fl} control mice in all experiments. The genetic background of each group was 129/Ola; BKW; C57Bl/6; SJLF₁. All procedures were carried out with the approval of the University of Glasgow and University of Edinburgh Local Ethical Review Committees, under Home Office Project and Personal Licence authority.

Hypobaric Chambers

EC ET_B^{-/-} mice and control mice (10 animals/group) were exposed to hypobaric hypoxic conditions for 14 days by housing them in a specially designed hypobaric chamber, as previously described [20]. The chamber was depressurised over the course of 2 days to 550 mbar [55 kPa or 413 mm Hg, equivalent to FiO₂ (percentage oxygen in inspired air) = 10%]. A further 10 age-matched mice of each genotype were maintained in the same room breathing air at atmospheric pressure (FiO₂ = 21%). All mice were allowed free access to standard rodent chow and water throughout the study and kept under 12-hour light/dark cycles.

Haemodynamic Studies

Anaesthesia was induced with 2–4% halothane and maintained with 1.5% halothane using a mix (1 part:3 parts) of NO₂ and high-flow O₂. Pressure and heart rate measurements were performed and analysed as previously described [25]. Systemic arterial pressure was measured via a cannula (Portex, 0.75 mm OD) inserted into the right carotid artery. A 25-gauge needle was advanced into the right ventricle (RV) via a transdiaphragmatic approach for measurement of right ventricular pressure (RVP). At the end of the experiment, mice were killed by lethal overdose of halothane. The heart was removed, blotted dry of blood and weighed. The right lung was placed in formal saline (1 part 37% formaline:9 parts 0.9% saline) for histology and third-order PAs dissected from the left lung for wire myography experiments.

Assessment of RV Hypertrophy

Hearts were dissected clean of pericardial tissue, blotted dry and the atria and great vessels removed to the plane of the atrioventricular valves. The RV free wall was dissected free from the left ventricle and septum (LV+S) and weighed. The ratios of RV/body weight (BW), RV/(LV+S) and RV/total ventricles (TV) were calculated [25].

Lung Histology

The right lung was embedded in paraffin and 10- μ m sections stained with Miller's elastin stain [26] and with picosirius red for collagen [27]. Sections were microscopically assessed for muscularisation of small PAs (25–100 μ m external diameter) associated with an airway distal to the respiratory bronchiole. Arteries were considered muscularised if they possessed a distinct double-elastic lamina visible for at least half the diameter of the vessel in cross-section (fig. 2). The percentage of vessels containing double-elastic lamina was calculated as the number of muscularised

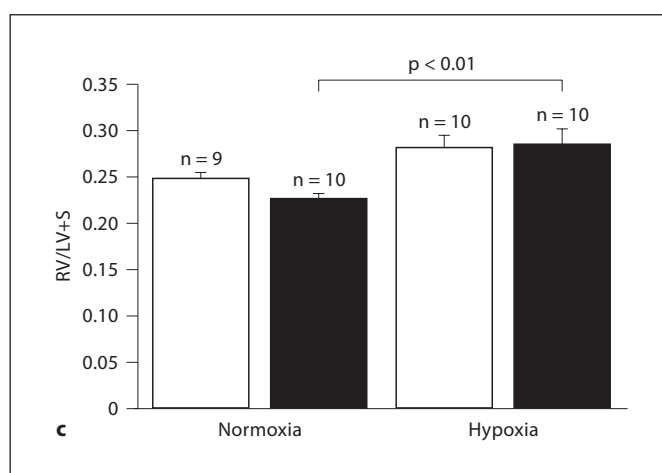
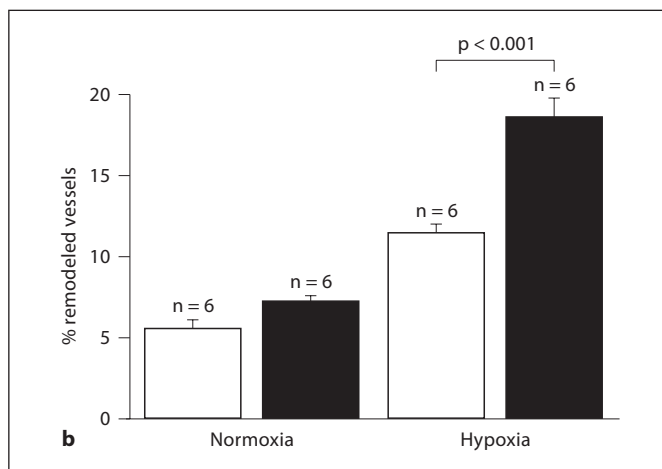
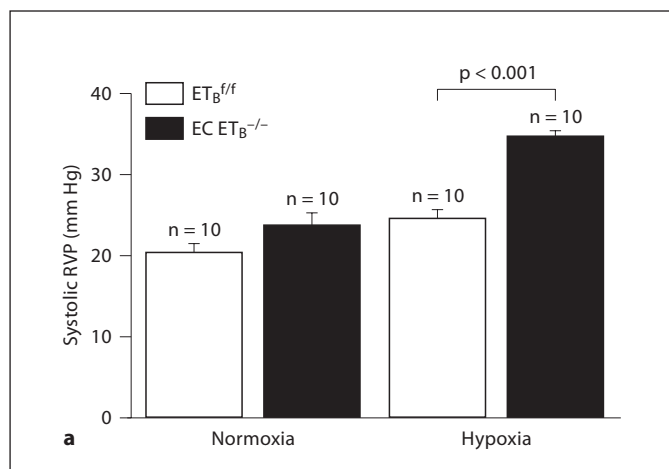


Fig. 1. **a** Systolic right ventricular pressure of anaesthetised ET_B^{ff/f} and EC ET_B^{-/-} mice (n = 10). **b** Percentage of muscularised vessels in lungs from EC ET_B^{-/-} mice and ET_B^{ff/f} controls (n = 6) following 14 days of normoxia or hypobaric hypoxia. **c** Right ventricular hypertrophy, as determined by the ratio RV/(LV+S), of ET_B^{ff/f} and EC ET_B^{-/-} mice (n = 10).

vessels/total number of vessels counted per section × 100. Three sections from each right lung were assessed for every mouse. A total of 6 mice per group were analysed.

In vitro Wire Myography

Third-order PAs (first interlobar; approx. 300 μm internal diameter) from the left lung were cut to yield two 2 mm-long segments, which were then mounted onto a wire myograph. Vessels were bathed in Krebs buffer solution (118.4 mM NaCl; 25 mM NaHCO₃; 4.7 mM KCl; 1.2 mM KH₂PO₄; 0.6 mM MgSO₄; 2.5 mM CaCl₂; 11 mM glucose; pH 7.4) at 37°C and constantly bubbled with 16% O₂/5% CO₂. Tension was applied to give transmural pressures equivalent to 12–14 mm Hg for controls and 30–33 mm Hg for hypoxic animals. These pressures are similar to those experienced by pulmonary vessels in vivo in rodents under similar hypobaric conditions [28, 29]. Following equilibration, PA rings were constricted twice with 50 mM KCl solution. Cumulative concentration-response curves were constructed for ET-1 (10⁻¹⁵ to 10⁻⁷ M) following a 30-min incubation with 100 μM N-nitro-L-arginine methylester (L-NAME) or vehicle. All responses were expressed as a percentage of the maximal KCl-induced constriction.

Drugs

Halothane, formalin and L-NAME were all purchased from Sigma-Aldrich (Gillingham, UK). ET-1 was purchased from Merck Chemicals Limited (Nottingham, UK).

Data Analysis and Statistical Procedures

Data are expressed as means ± SEM. Statistical comparisons were made by two-way ANOVA. When significance was attained (p < 0.05), differences were established using the Bonferroni multiple comparison test. In the myography studies, pEC₅₀ values were calculated from concentration-response curves by graphical interpolation (GraphPad Prism 4.0).

Results

Haemodynamic Studies

Systemic mean arterial blood pressure was not significantly different between EC ET_B^{-/-} mice and controls under either normoxic (ET_B^{ff/f} 99 ± 4 mm Hg; EC ET_B^{-/-}

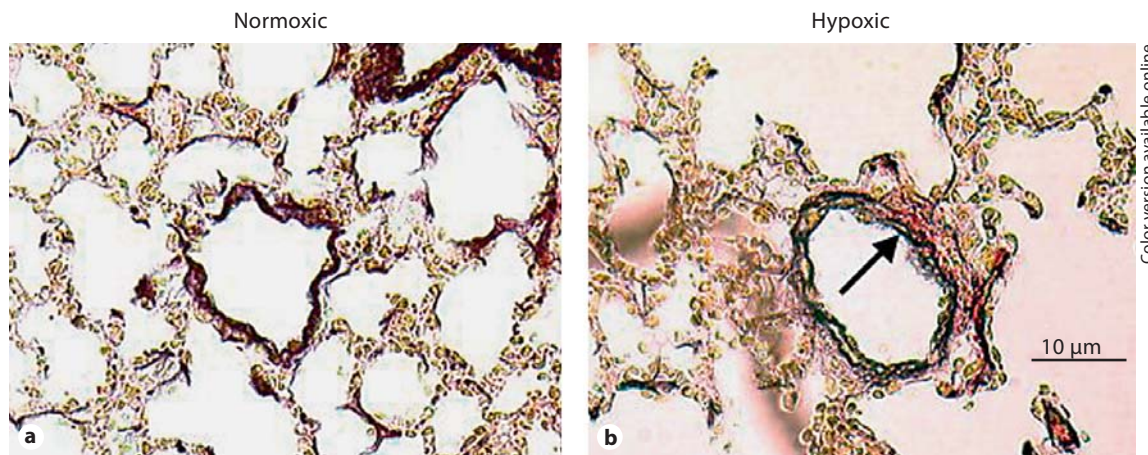


Fig. 2. Representative sections illustrating normal murine small PAs under normoxic conditions (a) and muscularised arteries with a double elastic lamina (b, arrow) that develop following 14 days of hypobaric hypoxia. The proportion of muscularised to normal vessels in response to hypoxia was greater in EC $ET_B^{-/-}$ mice than in $ET_B^{f/f}$ mice. Sections are stained with Miller's elastin stain and picosirius red collagen stain.

Table 1. Indices of right ventricular hypertrophy in $ET_B^{f/f}$ and EC $ET_B^{-/-}$ mice under normoxic conditions or following 14 days of hypobaric hypoxia

	$ET_B^{f/f}$			EC $ET_B^{-/-}$		
	RV/(LV+S)	RV/TV	RV/BW	RV/(LV+S)	RV/TV	RV/BW
Normoxic	0.25 ± 0.009	0.20 ± 0.006	0.80 ± 0.065	0.22 ± 0.009	0.18 ± 0.006	0.75 ± 0.047
Hypoxic	0.28 ± 0.012	0.22 ± 0.007	1.03 ± 0.083*	0.29 ± 0.017*	0.22 ± 0.010*	0.98 ± 0.045*

Values are means ± SEM (10 animals/group). * $p < 0.05$ for comparison between normoxic and hypoxic animals within each genotype.

101 ± 4 mm Hg) or hypoxic conditions ($ET_B^{f/f}$ 93 ± 4 mm Hg; EC $ET_B^{-/-}$ 103 ± 4 mm Hg), as previously reported [23]. No difference in heart rate was observed. Although systolic RVP was similar between genotypes under normoxic conditions, following 2 weeks of hypoxia, systolic RVP in EC $ET_B^{-/-}$ mice was significantly elevated compared to hypoxic $ET_B^{f/f}$ controls (fig. 1a).

Right Ventricular Hypertrophy

Relative right ventricular mass, as measured by RV/(LV+S) and RV/TV ratios, tended to be lower in normoxic EC $ET_B^{-/-}$ mice compared to normoxic $ET_B^{f/f}$ controls. Body weight fell by approximately 3.4 g in both genotypes following 2 weeks of hypoxia. Both genotypes demonstrated a significant increase in RV/BW ratio when exposed to hypoxia. However, only hypoxic EC $ET_B^{-/-}$ mice

demonstrated a significant increase in RV/(LV+S) ratio and RV/TV, suggesting a preferential increase in RV mass (fig. 1c; table 1).

Vascular Morphology

Under normoxic conditions, the percentage of muscularised vessels was similar in EC $ET_B^{-/-}$ and $ET_B^{f/f}$ mice. Following hypoxia, muscularisation was observed in a significantly greater proportion of vessels from EC $ET_B^{-/-}$ mice compared to controls (fig. 1b, 2).

Myography

Neither genotype nor FiO_2 influenced the E_{max} or pEC_{50} of PAs to ET-1 (table 1). Treatment with L-NAME did not significantly alter the maximum constriction or tissue sensitivity to ET-1 in either genotype (table 2).

Table 2. Potency and maximum effect of ET-1 in PA rings from EC ET_B^{-/-} and ET_B^{ff} mice

	ET _B ^{ff}			EC ET _B ^{-/-}		
	pEC ₅₀	E _{max}	n	pEC ₅₀	E _{max}	n
Normoxic	9.2 ± 0.1	121.9 ± 4.8	7	8.8 ± 0.1	109.7 ± 7.2	7
Hypoxic	10.1 ± 0.2	116.8 ± 9.0	5	9.6 ± 0.1	125.4 ± 5.5	5
Normoxic + L-NAME	9.7 ± 0.2	126.3 ± 3.4	7	9.1 ± 0.9	120.2 ± 5.0	6
Hypoxic + L-NAME	10.1 ± 0.2	124.3 ± 7.1	6	10.5 ± 0.1	143.0 ± 6.0	9

E_{max} is expressed as a percentage of the maximal contractile response to 50 mM KCl solution.

Discussion

This study demonstrates that in response to 14 days of hypobaric hypoxia, selective loss of EC ET_B results in an exaggerated increase in systolic RVP, an increase in RV mass and an increase in the proportion of muscularised small PAs. However, we did not observe systemic hypertension, implying a selective effect of loss of EC ET_B in the pulmonary vasculature.

After 14 days of hypoxia, we observed no increase in systolic RVP in control ET_B^{ff} mice that were of the same genetic background as EC ET_B^{-/-} mice. The development of hypoxia-induced PAH differs between mice of different strains, although even relatively resistant mice have been shown to develop raised systolic RVP after 4 weeks of hypoxia [30]. It is likely that our control ET_B^{ff} mice would have developed elevated systolic RVP after such a prolonged period of hypoxic exposure. However, we can conclude that loss of EC ET_B either accelerated the increase in systolic RVP in this strain or enabled PAH to develop in a strain resistant to the effects of hypoxia.

There are several possible mechanisms that may underlie the exaggerated increase in RVP in EC ET_B^{-/-} mice. First, EC ET_B vasodilator pathways are likely to be an important protective mechanism that limits the development of PAH during chronic hypoxia. In vitro studies of rat pulmonary microvascular ECs demonstrate that an increase in shear stress increases ET_B expression and enhances ET-1-mediated ET_B-dependent eNOS activation [31]. Lungs from rescued ET_B-deficient rats also demonstrate an exaggerated pressor response to ET-1 [32], due in part to reduced NO and PGI₂ production [17]. Studies of eNOS over-expressing [33] and eNOS KO mice [34, 35] have revealed that a reduction in EC-derived NO increases vascular tone and muscularisation of PAs. We have

previously reported endothelial dysfunction with decreased NO bioavailability in the absence of systemic hypertension in the aortae of EC ET_B^{-/-} mice [23]. Thus, impaired EC ET_B-mediated NO/PGI₂ release from pulmonary resistance vessels during hypoxia may have contributed to the development of PAH. We also observed an exaggerated increase in the number of muscularised small pulmonary vessels in EC ET_B^{-/-} mice following hypoxia. Thus, loss of ET_B-mediated NO release may also have had a permissive effect on hypoxia-induced vessel muscularisation.

Second, EC ET_B^{-/-} mice have elevated plasma ET-1 concentration [23] that may directly exert pressor and mitogenic actions to further promote the development of PAH. Studies in our laboratory demonstrate that clearance of ET-1 is impaired and plasma ET-1 increased approximately 4-fold in these mice [23]. Increased plasma ET-1 has also been reported in patients with PAH [6, 36, 37], though this may reflect increased production rather than impaired pulmonary clearance [38]. Loss of ET_B signalling has also been reported to increase ECE-1 mRNA expression [39], which may further contribute to an increase in ET-1. However, the limited experimental evidence available suggests that an isolated increase in ET-1 is insufficient to cause PAH. Under normoxic conditions, rats chronically infused with ET-1 by subcutaneous pump do not develop raised systolic RVP [40]. Similarly, transgenic mice that over-express preproET-1 have elevated plasma ET-1, exhibit pulmonary inflammation and fibrosis, but do not develop PAH, even when exposed to mild hypoxia (FiO₂ 16%) [41]. Thus, although an increase in ET-1 may contribute to many of the pathological processes associated with PAH, concomitant loss of NO/PGI₂-mediated vasodilator pathways is likely to be necessary for PAH to develop.

Although expression of Tie2 was thought to be exclusively restricted to ECs, Tie2-positive monocytes have now been identified [42, 43]. Thus, Cre-Lox-mediated ablation of the ET_B gene may have occurred in such inflammatory cells, as well as in ECs, potentially complicating the interpretation of the phenotype of the EC ET_B^{-/-} mice. Although no inflammatory infiltrate was seen in the lung sections from the hypoxic EC ET_B^{-/-} mice, further studies to clarify the role of such macrophages in the development of PAH are required.

We found no difference in the maximal response or sensitivity of 3rd order PAs to ET-1 in EC ET_B^{-/-} mice. The absence of any change in ET-1-mediated constriction following L-NAME suggests that NO does not contribute significantly to vascular tone in vessels of this size. This finding does not necessarily contradict our hypothesis that loss of ET_B-mediated NO release contributed to hypoxia-induced PAH in EC ET_B^{-/-} mice. Indeed, studies of rat intrapulmonary arteries following 4 h of either normoxia or hypoxia found similar results, demonstrating no effect of nitro-L-arginine on ET_B-mediated contraction [44]. In contrast, chronic in vivo telemetric measurements of PAP demonstrate that L-NAME acutely increases PAP in the mouse [45], suggesting that NO is an important determinant of PAP in this species. Thus, hypoxia-induced PAH in EC ET_B^{-/-} mice may be due to an increase in the tone of resistance arterioles smaller than those possible to study in myography experiments.

Several studies in mice report weight loss following exposure to hypoxia, an effect that is incompletely understood but may involve altered expression of genes regulated by hypoxia-inducible factor α [46]. However, we saw no difference in weight loss (approx. 3.4 g) between controls and EC ET_B^{-/-} mice. Although weight loss contributed to the increase in RV/BW ratio in both groups, we only observed an absolute increase in RV mass [as determined by RV/(LV+S) ratio and RV/TV ratio] in EC ET_B^{-/-} mice. This increase is, therefore, likely to reflect a true RV hypertrophic response to the increased PAP in EC ET_B^{-/-} mice, rather than any disproportionate weight loss in KOs.

In conclusion, this study indicates that EC ET_B play an important protective role during prolonged hypoxia that limits vascular remodelling and the development of pulmonary hypertension.

Acknowledgements

N.F.K. was a British Heart Foundation Junior Research Fellow (FS/03/006/15198). A.J.B. was funded by a Wellcome Trust Clinical Research Fellowship (055891/Z/98/Z/DG/MH/fh). This work was supported by the Wellcome Trust Cardiovascular Research Initiative (Grant No. 065901/Z/01/Z&A). Y.D. and I.M. were funded by the BBSRC and BHF, respectively.

References

- Humbert M, Barst RJ, Robbins IM, Channick RN, Galie N, Boonstra A, Rubin LJ, Horn EM, Manes A, Simonneau G: Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2. *Eur Respir J* 2004;24:353–359.
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–415.
- Stelzner TJ, O'Brien RF, Yanagisawa M, Sakurai T, Sato K, Webb S, Zamora M, McMurtry IF, Fisher JH: Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. *Am J Physiol* 1992;262:L614–L620.
- Frasch HF, Marshall C, Marshall BE: Endothelin-1 is elevated in monocrotaline pulmonary hypertension. *Am J Physiol* 1999;276:L304–L310.
- Nakanishi K, Tajima F, Nakata Y, Osada H, Tachibana S, Kawai T, Torikata C, Suga T, Takishima K, Aurues T, Ikeda T: Expression of endothelin-1 in rats developing hypobaric hypoxia-induced pulmonary hypertension. *Lab Invest* 1999;79:1347–1357.
- Cacoub P, Dorent R, Nataf P, Carayon A, Riquet M, Noe E, Piette JC, Godeau P, Gandjbakhch I: Endothelin-1 in the lungs of patients with pulmonary hypertension. *Cardiovasc Res* 1997;33:196–200.
- Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G: Treatment of pulmonary arterial hypertension with the selective endothelin-A receptor antagonist sitaxsentan. *J Am Coll Cardiol* 2006;47:2049–2056.
- Firth JD, Ratcliffe PJ: Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. *J Clin Invest* 1992;90:1023–1031.
- Li H, Chen SJ, Chen YF, Meng QC, Durand J, Oparil S, Elton TS: Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. *J Appl Physiol* 1994;77:1451–1459.
- Davie N, Haleen SJ, Upton PD, Polak JM, Yacoub MH, Morrell NW, Wharton J: ET_A and ET_B receptors modulate the proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Crit Care Med* 2002;165:398–405.
- Takahashi H, Soma S, Muramatsu M, Oka M, Fukuchi Y: Upregulation of ET-1 and its receptors and remodeling in small pulmonary veins under hypoxic conditions. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1104–L1114.

- 13 Soma S, Takahashi H, Muramatsu M, Oka M, Fukuchi Y: Localization and distribution of endothelin receptor subtypes in pulmonary vasculature of normal and hypoxia-exposed rats. *Am J Respir Cell Mol Biol* 1999; 20:620–630.
- 14 de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, Vane JR: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelin-derived relaxing factor. *Proc Natl Acad Sci USA* 1988; 85:9797–9800.
- 15 Takayanagi R, Kitazumi K, Takasaki C, Ohnaka K, Aimoto S, Tasaka K, Ohashi M, Nawata H: Presence of non-selective type of endothelin receptor on vascular endothelium and its linkage to vasodilation. *FEBS Lett* 1991; 282:103–106.
- 16 Nishida M, Okada Y, Akiyoshi K, Eshiro K, Takoaka M, Garipey CE, Yanagisawa M, Matsumura Y: Role of endothelin ETB receptor in the pathogenesis of monocrotaline-induced pulmonary hypertension in rats. *Eur J Pharmacol* 2004; 496:159–165.
- 17 Ivy DD, Yanagisawa M, Garipey CE, Gebb SA, Colvin KL, McMurtry IF: Exaggerated hypoxic pulmonary hypertension in endothelin B receptor-deficient rats. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L703–L712.
- 18 Dupuis J, Goresky CA, Fournier A: Pulmonary clearance of circulating endothelin-1 in dogs in vivo: exclusive role of ETB receptors. *J Appl Physiol* 1996; 81:1510–1515.
- 19 McCulloch KM, Docherty CC, Morecroft I, MacLean MR: Endothelin B receptor-mediated contraction in human pulmonary resistance arteries. *Br J Pharmacol* 1996; 119:1125–1130.
- 20 McCulloch KM, Docherty C, MacLean MR: Endothelin receptors mediating contraction of rat and human pulmonary resistance arteries: effect of chronic hypoxia in the rat. *Br J Pharmacol* 1998; 123:1621–1630.
- 21 Balyakina EV, Chen D, Lawrence ML, Manning S, Parker RE, Shappell SB, Meyrick B: ET-1 receptor gene expression and distribution in L1 and L2 cells from hypertensive sheep pulmonary artery. *Am J Physiol Lung Cell Mol Physiol* 2002; 283:L42–L51.
- 22 Black SM, Mata-Greenwood E, Dettman RW, Ovadia B, Fitzgerald RK, Reinhartz O, Thelitz S, Steinhorn RH, Gerrets R, Hendricks-Munoz K, Ross GA, Bekker JM, Jøhengen MJ, Fineman JR: Emergence of smooth muscle cell endothelin B-mediated vasoconstriction in lambs with experimental congenital heart disease and increased pulmonary blood flow. *Circulation* 2003; 108:1646–1654.
- 23 Bagnall AJ, Kelland NF, Gulliver-Sloan F, Davenport AP, Gray GA, Yanagisawa M, Webb DJ, Kotelevtsev YV: Deletion of endothelial cell endothelin B receptors does not affect blood pressure or sensitivity to salt. *Hypertension* 2006; 48:286–293.
- 24 Kisanuki YY, Hammer RE, Miyazaki J, Williams SC, Richardson JA, Yanagisawa M: Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. *Dev Biol* 2001; 230:230–242.
- 25 Keegan A, Morecroft I, Smillie D, Hicks MN, MacLean MR: Contribution of the 5-HT_{1B} receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT_{1B}-receptor knockout mice and the 5-HT_{1B/1D}-receptor antagonist GR127935. *Circ Res* 2001; 89:1231–1239.
- 26 Miller PJ: An elastin stain. *Med Lab Technol* 1971; 28:148–149.
- 27 Junqueira LC, Montes GS, Krisztan RM: The collagen of the vertebrate peripheral nervous system. *Cell Tissue Res* 1979; 202:453–460.
- 28 Herget J, Palecek F: Experimental chronic pulmonary hypertension. *Int Rev Exp Pathol* 1978; 18:347–406.
- 29 MacLean MR, Deuchar GA, Hicks MN, Morecroft I, Shen S, Sheward J, Colston J, Loughlin L, Nilsen M, Dempsey Y, Harmor A: Overexpression of the 5-hydroxytryptamine transporter gene: effect on pulmonary hemodynamics and hypoxia-induced pulmonary hypertension. *Circulation* 2004; 109:2150–2155.
- 30 Tada Y, Laudi S, Harral J, Carr M, Ivester C, Tanabe N, Takiguchi Y, Tatsumi K, Kuriyama T, Nichols WC, West J: Murine pulmonary response to chronic hypoxia is strain specific. *Exp Lung Res* 2008; 34:313–323.
- 31 Tang L, Luo B, Patel RP, Ling Y, Zhang J, Fallon MB: Modulation of pulmonary endothelial endothelin B receptor expression and signaling: implications for experimental hepatopulmonary syndrome. *Am J Physiol Lung Cell Mol Physiol* 2007; 292:L1467–L1472.
- 32 Ivy D, McMurtry IF, Yanagisawa M, Garipey CE, Le Cras TD, Gebb SA, Morris KG, Wiseman RC, Abman SH: Endothelin B receptor deficiency potentiates ET-1 and hypoxic pulmonary vasoconstriction. *Am J Physiol Lung Cell Mol Physiol* 2001; 280:L1040–L1048.
- 33 Ozaki M, Kawashima S, Yamashita T, Ohashi Y, Rikitake Y, Inoue N, Hirata KI, Hayashi Y, Itoh H, Yokoyama M: Reduced hypoxic pulmonary vascular remodeling by nitric oxide from the endothelium. *Hypertension* 2001; 37:322–327.
- 34 Studel W, Scherrer-Crosbie M, Bloch KD, Weimann J, Huang PL, Jones RC, Picard MH, Zapol WM: Sustained pulmonary hypertension and right ventricular hypertrophy after chronic hypoxia in mice with congenital deficiency of nitric oxide synthase 3. *J Clin Invest* 1998; 101:2468–2477.
- 35 Fagan KA, Fouty BW, Tyler RC, Morris KG Jr, Hepler LK, Sato K, LeCras TD, Abman SH, Weinberger HD, Huang PL, McMurtry IF, Rodman DM: The pulmonary circulation of homozygous or heterozygous eNOS-null mice is hyperresponsive to mild hypoxia. *J Clin Invest* 1999; 103:291–299.
- 36 Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ: Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; 328:1732–1739.
- 37 Stewart DJ, Levy RD, Cernacek P, Langleben D: Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med* 1991; 114:464–469.
- 38 Langleben D, Dupuis J, Langleben I, Hirsch AM, Baron M, Senecal JL, Giovino M: Etiology-specific endothelin-1 clearance in human precapillary pulmonary hypertension. *Chest* 2006; 129:689–695.
- 39 Naomi S, Iwaoka T, Disashi T, Inoue J, Kanezaka Y, Tokunaga H, Tomita K: Endothelin-1 inhibits endothelin-converting enzyme-1 expression in cultured rat pulmonary endothelial cells. *Circulation* 1998; 97:234–236.
- 40 Migneault A, Sauvageau S, Villeneuve L, Thorin E, Fournier A, Leblanc N, Dupuis J: Chronically elevated endothelin levels reduce pulmonary vascular reactivity to nitric oxide. *Am J Respir Crit Care Med* 2005; 171:506–513.
- 41 Hoher B, Schwarz A, Fagan KA, Thone-Reineke C, El-Hag K, Kusserow H, Elitok S, Bauer C, Neumayer HH, Rodman DM, Theuring F: Pulmonary fibrosis and chronic lung inflammation in ET-1 transgenic mice. *Am J Respir Cell Mol Biol* 2000; 23:19–26.
- 42 De Palma M, Venneri MA, Roca C, Naldini L: Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat Med* 2003; 9:789–795.
- 43 De Palma M, Venneri MA, Galli R, Sergi L, Politi LS, Sampaoli M, Naldini L: Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005; 8:211–226.
- 44 Wang X, Tong M, Chinta S, Raj JU, Gao Y: Hypoxia-induced reactive oxygen species downregulate ETB receptor-mediated contraction of rat pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol* 2006; 290:L570–L578.
- 45 Schwenke DO, Pearson JT, Mori H, Shirai M: Long-term monitoring of pulmonary arterial pressure in conscious, unrestrained mice. *J Pharmacol Toxicol Methods* 2006; 53:277–283.
- 46 Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, Semenza GL: Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α . *J Clin Invest* 1999; 103:691–696.