Peptides. Author manuscript; available in PMC 2013 July 03.

Published in final edited form as:

Peptides. 2008 November ; 29(11): 2039–2045. doi:10.1016/j.peptides.2008.08.003.

Endothelin-3-dependent pulmonary vasoconstriction in monocrotaline-induced pulmonary arterial hypertension

Stéphanie Sauvageau[a], **Eric Thorin**[a,b], **Louis Villeneuve**[a], and **Jocelyn Dupuis**[a,c,*]

[a]Research Center, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada

[b]Department of Surgery, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada

[c]Department of Medicine, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada

Abstract

Blockade of the endothelin (ET) system is beneficial in pulmonary arterial hypertension (PAH). The contribution of ET-3 and its interactions with ET receptors have never been evaluated in the monocrotaline (MCT)-induced model of PAH. Vasoreactivity of pulmonary arteries was investigated; ET-3 localization was determined by confocal imaging and gene expression of prepro-ET-3 quantified using RT-PCR. ET-3 plasma levels tended to increase in PAH. ET-3 localized in the media of pulmonary arteries, where gene expression of prepro-ET-3 was reduced in PAH. ET-3 induced similar pulmonary vasoconstrictions in sham and PAH rats. In sham rats, the ET_A antagonist A-147627 (10 nmol/l) significantly reduced the maximal response to ET-3 (E_{max} 77 ± 1 to 46 ± 2%, mean ± S.E.M., $P < 0.001$), while the ET_B antagonist A-192621 (1 μmol/l) reduced the sensitivity (EC_{50} 21 ± 7 to 59 ± 16 nmol/l, $P < 0.05$) without affecting E_{max}. The combination of both antagonists completely abolished ET-3-induced pulmonary vasoconstriction. In PAH, the ET_A antagonist further reduced the maximal response to ET-3 and shifted the EC_{50} (E_{max} 23 ± 2%, $P < 0.001$, EC_{50} 104 ± 24 nmol/l, $P < 0.05$), while the ET_B antagonist only shifted the EC_{50} (123 ± 36 nmol/l, $P < 0.05$) without affecting the E_{max}. In PAH, dual ET receptor inhibition did not further reduce constriction compared to selective ET_A inhibition. ET-3 significantly contributes to pulmonary vasoconstriction by activating the ET_B at low concentration, and the ET_A at high concentration. The increased inhibitory effect of the ET_A antagonist in PAH suggests that the contribution of ET_B to ET-3-induced vasoconstriction is reduced. Although ET-3 is a potent pulmonary vasoconstrictor in PAH, its potential pathophysiologic contribution remains uncertain.

Keywords

Endothelial receptors; Endothelins; Pulmonary circulation; Vasoactive agents

*Corresponding author at: Research Center, Montreal Heart Institute, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada. Tel.: +1 514 376 3330; fax: +1 514 376 1355. jocelyn.dupuis@bellnet.ca (J. Dupuis).

1. Introduction

Endothelins are a family of three related peptides. Mammals, including humans, produce three distinct isoforms of the ET peptide family, ET-1, ET-2 and ET-3, which may have different profiles of biological activity on vascular and non-vascular tissues [11]. These three isopeptides show different pharmacological profiles of pressor/vasoconstrictor activities [11]. ET-3 differs from ET-1 by six amino acid substitutions. ET-3 is highly expressed in the brain, but is also present in the gut, pancreas, liver, kidneys and lungs. Two receptor subtypes for ET have been identified in mammals. The ET_A recognizes ET-1 with high affinity and ET-3 with low affinity [2]. The ET_B recognizes the different ET with similar and high affinity [20].

Among the known physiological properties of ET-3 is its interaction with ET_B that is crucial for the development of melanocytes [1]. Moreover, the expression of ET-3 by the mesenchymal cells of the colon is necessary for successful development of the distal enteric nervous system [3,10]. Elevated plasma ET-3 levels have been demonstrated in patients undergoing haemodialysis [23], in patients with cirrhosis [24] and glomerulonephritis [25].

The ET system is activated in pulmonary arterial hypertension (PAH) [14] and ET receptor antagonists (ERA) have demonstrated their effectiveness and have been approved for the therapy of this condition [9]. A recent study by Montani et al. has demonstrated for the first time that ET-3 plasma concentration decreased in patients suffering from PAH with various ethiology and that these levels correlated with clinical parameters of severity of PAH. Moreover, they have demonstrated that the ratio ET-1/ET-3 might bring more information than the measurement of ET-1 alone [17]. Although the deleterious effects of ET-1 on the pulmonary circulation are well established, only few studies have focused on ET-3 in PAH. Therefore, the purpose of this study was to evaluate the potential implication of ET-3 in MCT-induced PAH and evaluate the roles of ET_A and ET_B on ET-3-induced pulmonary vascular reactivity. We have: (1) measured ET-3 levels in both plasma and whole lung tissue homogenates; (2) investigated ET-3 localization in pulmonary vessels using confocal imaging; (3) measured gene expression of preproET-3 in pulmonary resistance arteries; (4) evaluated the roles of ET_A and ET_B in ET-3-induced vasoconstriction of pulmonary resistance arteries.

2. Experimental/materials and methods

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). This study was approved by the Animal Research Committee of the Montreal Heart Institute and conducted according to the guidelines from the Canadian Council of Animal Care.

2.1. Monocrotaline-induced PAH

Male Wistar rats weighting between 300 and 350 g received an intraperitoneal (IP) injection of either 0.5 ml 0.9% saline or 0.5 ml 60 mg/kg monocrotaline (MCT) [6,12,19]. Five weeks later, rats were anesthetized for hemodynamic measurements as previously described in

detail [16]. Briefly, the right jugular vein and right carotid artery were isolated, incised and cannulated with Millar catheters to measure central venous, right ventricular, systemic arterial and left ventricular pressures. The right inferior lobe was then snap frozen for either confocal imaging or ET-3 quantification. The pulmonary resistance arteries were harvested for vascular reactivity or snap frozen for RNA extraction.

2.2. ET-3 levels

Plasma and pulmonary tissue homogenate samples were passed on Sep-Pak C_{18} columns (Waters, Milford, MA, USA) before determination of ET-3 levels by ELISA according to the manufacturer's instructions (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan). The ET-3 antibody used was directed primarily towards ET-3 with 7.5% cross-reactivity with ET-1. Cross-reactivity for ET-1 (1–31) and ET-2 (1–31) were 0.1% each. The intra and interassay coefficient was 7.1 and 3.4%, respectively.

2.3. Quantification of gene expression of preproET-3 by real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from small intralobar pulmonary arteries from the pulmonary right inferior lobe using an RNeasy mini-kit (Qiagen Inc., Mississauga, ON, Canada). The reverse transcriptase reaction contained 5 ng/μL total RNA (each sample), M-MLV reverse transcriptase (800 U, Invitrogen, Burlington, ON, Canada), RNAseOUT (40 U, Invitrogen, Burlington, ON, Canada), anti-sens primer (4 pM, Invitrogen, Burlington, ON, Canada), dNTPs (0.5 mmol/l, MBI Fermentas, Burlington, ON, Canada), and supplied optimal buffers. The reaction protocol consisted of 3 successive incubation steps: (1) 25 °C for 10 min; (2) 37 °C for 50 min; and (3) 70 °C for 15 min. RT-PCR was performed with 1 ng of cDNA template containing the appropriate primer concentration; preproET-3 (300 nmol/l) and SYBR Green PCR master mix (Applied BioSystems, Foster City, CA, USA). Primers for each gene were obtained from distinct exons that spanned an intron by using the Ensembl Genome Browser program (http://www.ensembl.org). The sequence specificity of each primer was verified with the Blast program derived from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The primers used were as follows:

Rat preproET-3	Forward	5′-GGGACCAGGAGGAAAAGAGGGTG-3′
	Reverse	5′-ACTGGGAACTTTCTGGAACTGG-3′
Rat cyclophiline A	Forward	5′-AGGTCCTGGCATCTTGTC-3′
	Reverse	5′-TGATCTTCTTGCTGGTCT-3′

For each primer set, the PCR conditions were optimized to obtain only the specific product with an efficiency calculated from dilution curves of between 95 and 105%. Each sample was measured in duplicate and each plate contained negative and positive controls. PCR product was purified, sequenced and confirmed to be the gene of interest. Cyclophiline A was chosen as the house keeping gene which did not change with MCT treatment.

2.4. ET-3 staining in small pulmonary arteries

Immunofluorescence and confocal imaging were performed as recently described in detail [15]. Briefly the lungs were oriented to cross-section the arteries of interest and 8 μmol/l cryocuts were performed. ET-3 antibody (1/200, rabbit, Phoenix Pharmaceuticals, Belmont, CA, USA) was incubated with alpha smooth muscle actin antibody (1/200, mouse, Sigma, St-Louis, MO, USA). Anti-rabbit Alexa 555 antibody (1/800, donkey, Molecular Probes, Burlington, ON, Canada) and anti-mouse Alexa 647 antibody (1/800, donkey, Molecular Probes) antibodies were diluted in their respective antibody diluents and applied. In order to identify the limits of the media and of the endothelium we used the internal elastic lamina (IEL) and external elastic lamina (EEL) auto-fluorescence.

2.5. Confocal imaging and deconvolution

Slides were analyzed using a Zeiss LSM 510 confocal microscope. We used a plan Apo-Chromat 63×/1.4 oil DIC objectif. HeNel (543 nm) and HeNe2 (633 nm) lasers were used for excitation of the anti-rabbit Alexa 555 and anti-mouse Alexa 647 antibodies, respectively. IEL and EEL auto fluorescence was obtained with the Argon laser line (488 nm) and collected between 505 and 530 nm. Z-stacks of each tissues were performed and images were taken at every 0.8 μmol/l (top to bottom) in order to respect the Nyquist criteria in z-sampling. Z-stacks were *deconvolved* using the Maximum Likelihood Estimation (MLE) algorithm of the Huygens Pro software (Version 2.4.1, Scientific Volume Imaging). Transparent projections (in face view) were applied to each z-stack using the Projection tool of the LSM 510 software.

2.6. Vascular reactivity studies

Experiments were conducted on fifth generation pulmonary arteries of 1–2 mm long and 150–200 μm diameters using a micro-vascular myograph. Approximately five to six arteries were harvested from each animal. Only one reactivity protocol per artery coming from different animals was undertaken such that for each stimulation protocol, the number of arteries corresponds to the number of different animal studied. They were set up on a myograph with a resting tension of 80–100 mg and bathed in a physiological salt solution (PSS) containing the following composition (mmol/l): NaCl 119; KCl 4.7; KH_2PO_4 1.18; $MgSO_4$ 1.17;$NaHCO_3$ 1.17;$CaCl_2$ 1.16;EDTA0.023;glucose10.The arteries were equilibrated for 30 min at 37 °C in PSS and bubbled with 5% CO_2, 12% O_2 and balance in N_2. For each vessel, the integrity of the endothelium was determined by testing endothelium-dependent vaso-relaxation to acetylcholine (100 μmol/l). The general contractile capacities of pulmonary arteries from both groups were evaluated with: a high potassium solution (127 mM), a concentration response curve toET-3 and ET-1(0.1 nmol/l to 0.3 μmol/l) in both absolute values (mg of tension) and as percentage of the maximal vasoconstriction (127 mM KCl). The ET-3 concentration–response curve was assessed in the presence and absence of an ET_A antagonist (A-147627, 10 nmol/l, ET_A:ET_B ~1800:1), an ET_B antagonist (A-192621, 1 μmol/l, ET_A:ET_B ~1:1400) or the combination of both antagonists. The concentrations of antagonists that were used in these experiments were based on previous experiments [20].

2.7. Drugs

The MCT was purchased from Sigma Chemical Co. The MCT was dissolved in 1.0N HCl, and the pH was adjusted to 7.4 with 1.0N NaOH. ET-3 was purchased from American Peptide (Sunnyvale, CA, USA). The ET_A-R antagonist A-147627 and ET_B-R antagonist A-192621 were kindly provided by Abbott Laboratories (USA).

2.8. Statistical analysis

All values are expressed as mean ± S.E.M. For each pharmacological condition on each isolated artery, the isometric recording of the concentration–response curves were fitted using a five-parameter logistic fit to determine the maximal responses as well as the EC_{50} values. At the end of the protocol, the maximal vasoconstriction was determined by changing the PSS with a high potassium solution (127 mmol/l KCl). ET-3-induced vasoconstrictions are expressed as a percentage of the maximal response. The differences between groups were evaluated with an unpaired student *t*-test. Statistical significance was assumed when $P < 0.05$.

3. Results

3.1. Effect of MCT treatment

Five weeks following the injection of MCT, the animals developed severe PAH as evidenced by higher right ventricular systolic pressure (77 ± 3 mmHg, $n = 58$, *vs.* 26 ± 1 mmHg, $n = 74$, $P < 0.001$) and the presence of right ventricular hypertrophy as measured from the ratio of right over left ventricular weight (0.64 ± 0.013 *vs.* 0.27 ± 0.004, $P < 0.001$).

3.2. ET-3 levels

As shown in Fig. 1A, plasma ET-3 levels tended to increase in PAH rats (0.96 ± 0.09 pg/ml, $n = 20$, $P = 0.0549$) when compared to sham rats (0.81 ± 0.09 pg/ml, $n = 19$). We found an increase ET-1/ET-3 plasma ratio between sham (4.09 ± 0.34, mean ± S.E.M.) and MCT rats (6.16 ± 0.72, $P < 0.05$). There were no correlation between ET-3 levels and the severity of pulmonary hypertension ($R^2 = 0.01$, $P = 0.65$) or with right ventricular hypertrophy ($R^2 = 0.08$, $P = 0.24$). Fig. 1B shows that ET-3 pulmonary tissue levels were significantly reduced in PAH (0.31 ± 0.04 pmol/l/mg of protein, $n = 20$, $P < 0.05$) when compared to sham rats (0.43 ± 0.04 pmol/l/mg of protein, $n = 19$).

3.3. Quantification of gene expression of preproET-3 in pulmonary resistance arteries

The preproET-3 mRNA levels in pulmonary resistance arteries were reduced in PAH (0.82 ± 0.10 dRn, $P < 0.05$) compared to sham animals (1.20 ± 0.10 dRn; Fig. 1C).

3.4. Immunofluorescence of ET receptors in small pulmonary arteries

Examples of composite Z-stack images obtained with the ET-3 and alpha smooth muscle actin antibodies are shown in Fig. 2. Auto-fluorescence of the IEL and EEL enables easy delineation of the endothelium. ET-3 immunostaining is only evident in the media of pulmonary resistance arteries from both sham (Fig. 2A) and PAH rats (Fig. 2B).

3.5. Vascular reactivity studies

The maximal depolarization induced by a high K^+ solution was significantly reduced in MCT rats (279 ± 16 mg, $P < 0.01$) when compared to sham rats (377 ± 20 mg). Similarly, the response induced by ET-3 and ET-1 was also significantly reduced in MCT rats (223 ± 10 mg, $P < 0.001$, 350 ± 10 mg, $P < 0.001$) when compared to sham rats (406 ± 14, 560 ± 8 mg). However, when expressed as the percentage of the maximal vasoconstriction induced by high K^+, the vasoconstriction induced by ET-3 is preserved in MCT rats (E_{max} 68 ± 2%, EC_{50} 33 ± 9 nmol/l; Fig. 3B) when compared to sham rats (E_{max} 77 ± 1%, EC_{50} 21 ± 7 nmol/l; Fig. 3A). In sham animals, the ET_A antagonist greatly reduced the maximal response to ET-3 (E_{max} 46 ± 2%, $P < 0.001$, EC_{50} 41 ± 29 nmol/l), while the ET_B antagonist shifted the EC_{50} without affecting E_{max} (E_{max} 68 ± 2%, EC_{50} 59 ± 16 nmol/l, $P < 0.05$). The combination of both antagonists, to achieve dual ET receptor blockade, completely abolished ET-3-induced vasoconstriction ($P < 0.001$). In PAH, the effect of ET_A inhibition was increased, with a marked reduction of the maximal response and a shift of the EC_{50} (E_{max} 23 ± 2%, $P < 0.001$, EC_{50} 104 ± 24 nmol/l, $P < 0.05$). In arteries isolated from PAH animals, inhibition of ET_B only shifted the EC_{50} without affecting the maximal response (E_{max} 52 ± 3%, EC_{50} 123 ± 36 nmol/l, $P < 0.05$). However, dual ET receptor blockade did not further reduce constriction compared to ET_A inhibition alone (E_{max} 23 ± 7%, $P < 0.001$, EC_{50} 36 ± 21 nmol/l).

4. Discussion

Among the isoforms of ET, ET-1 is generally considered the most important in the pathogenesis of PAH. Although the predominant ET isoform in the lungs is ET-1, there is also evidence of ET-3 expression, while that of ET-2 is non-detectable [7]. Both ET-1 and ET-3 induce constriction of pulmonary resistance arteries from humans and rats preparations [2] and both are able to stimulate lung fibroblasts [18]. These previous data would suggest the ET-3 as well as ET-1 could be implicated in the development of PAH. The interactions of ET-3 with ET receptors and their modification in the MCT model of PAH have never been evaluated. We therefore studied the implication of ET-3 in this model of PAH.

Following MCT treatment, there is a slight non-significant increase in ET-3 plasma levels when compared to controls while pulmonary tissue levels are reduced, suggesting that the increased plasma levels may not originate from the lungs. This is supported by our finding of a reduction in preproET-3 mRNA levels in pulmonary resistance vessels of MCT rats. Miyauchi et al. [16] have demonstrated that following MCT treatment, ET-1 levels are increased in plasma while they are reduced in the lungs. They suggested that the increase in plasma levels may originate from other organ such as the kidney and the heart. We would like to propose likewise, that the increase plasma levels of ET-3 may originate from other organs. This may be associated with a reduced clearance of ET-3 from the plasma, as we demonstrated that pulmonary ET clearance is reduced in this model of PAH [6]. Previous studies have shown that porcine aortic endothelial cells do not produce ET-3 [4]. Our results using confocal imaging are in agreement and demonstrate that ET-3 is mainly localized in the smooth muscle layer of pulmonary resistance arteries of both control and MCT rats.

Therefore, ET-3 is likely produced by a specific endothelin converting enzyme localized in the smooth muscle cells [8] and exerts its effects locally.

Here, we demonstrate that the overall reactivity of pulmonary resistance arteries is reduced in PAH as evidenced by a reduced response to maximal depolarization by a high K^+ solution. As illustrated in Fig. 2, with the alpha smooth muscle staining (blue), pulmonary arteries from MCT treated rats undergo an important remodeling process, which leads to a thicker media and therefore could potentially explain the reduced overall reactivity of pulmonary arteries. However, the vasoconstriction induced by ET-3 (when expressed as a percentage of the maximal vasoconstrictive capacity of the artery) is preserved in MCT rats. We also evaluated the contribution of ET_A and ET_B during ET-3-induced constriction of pulmonary vessels. In sham rats, the ET_B antagonist reduced the sensitivity to ET-3 without affecting the maximal response. These results suggest that at low concentration ET-3 preferentially binds to ET_B. The ET_A antagonist however, greatly reduced the maximal response without modifying the sensitivity to ET-3 suggesting that at high concentration ET-3 is able to bind to ET_A. Not surprisingly, the combination of both antagonists completely abolished the ET-3-induced pulmonary vasoconstriction. This is different from ET-1-induced vasoconstriction, which is entirely dependent on interactions between both receptor subtypes since the use of either the selective ET_A antagonist or the ET_B antagonist alone and at similar concentrations, had no significant effect on the ET-1 response [21]. This difference may be the result of a greater affinity of the ET_B receptor for ET-3. In PAH, the ET_A antagonist was able to reduce ET-3-induced contraction that was comparable to the combination of both antagonists, suggesting a reduction of the ET_B-dependent effect. In agreement, we previously reported that the balance of ET_B to ET_A receptors was altered in MCT rats, with a trend toward a lower percentage of ET_B when compared to control rats [12]. Thus, the increased inhibitory effect of the ET_A antagonist in PAH could partially be ascribed to the reduced expression of ET_B, revealing a predominant role of ET_A in ET-3-induced pulmonary vasoconstriction. Others such as McCullogh et al. have previously evaluated ET-3-induced pulmonary vasoconstriction in PAH rats induced by chronic hypoxia [13]. Similarly, they found no modification of the ET-3-induced vasoconstriction of pulmonary arteries following chronic hypoxia. Moreover, Shi et al. have also evaluated ET-3-induced pulmonary vasoconstriction in the post-obstructive pulmonary vasculopathy model using explanted lung tissues [22]. Although the methodological approach was substantially different, they found an increase ET-3-induced pulmonary vasoconstriction in their model that was associated with a probable reduction in ET_B receptor-mediated effects.

Although it is well accepted that activation of the endothelin system contributes to the pathophysiology of PAH, the possible role of ET-3 in this pathologic process is currently unknown. Increased plasma levels of ET-1 are detected in patients with various forms of PAH [14] and in various experimental models [12,16] and, based on the effectiveness of ET receptor antagonists it is generally accepted that ET-1 contributes to the disease process. However, few studies have evaluated ET-3 levels in human PAH. In patients with valvular heart disease and PH, ET-3 levels, like in the current study, were not elevated [5]. In a recent study, Montani et al. measured plasma ET-3 levels in 33 PAH subjects in comparison to 9 controls. They found that plasma ET-3 levels were significantly reduced and interestingly, that increased ET-1/ET-3 ratio seemed to be a prognostic factor in human PAH [17]. The

current pre-clinical study further demonstrates the complex interplay between endothelin isoforms and their receptors in normal and disease states. The exact role of ET-3 and its modifications in various forms of PAH will require further experimentation.

5. Conclusion

This study demonstrates a potential implication of another member of the ET family in PAH. In physiological conditions, we have demonstrated that at low concentration ET-3 preferentially binds to ET_B, although at high concentration it can also bind ET_A. Combination of both ET_A and ET_B antagonists is necessary to completely abolish ET-3-induced pulmonary vasoconstriction. Following MCT treatment and the development of PAH, the use of the ET_A antagonist by itself is sufficient to reduce the ET-3-dependent response as efficiently as the combination of both antagonists. Our results demonstrate that although ET-3 is a potent pulmonary vasoconstrictor in PAH, its potential pathophysiologic contribution remains uncertain.

6. Limitation of this study

As usual, the salient findings of this pre-clinical study cannot be readily extrapolated to human PAH, as this animal model may not reproduce the pathologic modifications of pulmonary vessels found in the various forms of human PAH.

Acknowledgments

This work was supported by the Canadian Institutes for Health Research, the Heart and Stroke Foundation of Canada and the Fondation de l'Institut de Cardiologie de Montréal. Dr. Jocelyn Dupuis is a National Researcher of the Quebec foundation for Health Research. Stéphanie Sauvageau is supported by a scholarship from the Heart and Stroke Foundation of Canada.

Abbreviations

PAH	pulmonary arterial hypertension
PH	pulmonary hypertension
EEL	external elastic lamina
IEL	internal elastic lamina
ET	endothelins
ERA	ET receptor antagonists
MCT	monocrotaline
PSS	physiological salt solution

References

1. Aoki H, Motohashi T, Yoshimura N, Yamazaki H, Yamane T, Panthier JJ, et al. Cooperative and indispensable roles of endothelin 3 and KIT signalings in melanocyte development. Dev Dyn. 2005; 233:407–17. [PubMed: 15768389]

2. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature. 1990; 348:730–2. [PubMed: 2175396]
3. Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, et al. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell. 1994; 79:1277–85. [PubMed: 8001160]
4. Bloch KD, Eddy RL, Shows TB, Quertermous T. cDNA cloning and chromosomal assignment of the gene encoding endothelin 3. J Biol Chem. 1989; 264:18156–61. [PubMed: 2509452]
5. Chang H, Wu GJ, Wang SM, Hung CR. Plasma endothelin levels and surgically correctable pulmonary hypertension. Ann Thorac Surg. 1993; 55:450–8. [PubMed: 8431058]
6. Dupuis J, Jasmin JF, Prie S, Cernacek P. Importance of local production of endothelin-1 and of the ET(B)receptor in the regulation of pulmonary vascular tone. Pulm Pharmacol Ther. 2000; 13:135–40. [PubMed: 10873551]
7. 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; 90:1023–31. [PubMed: 1522210]
8. Hasegawa H, Hiki K, Sawamura T, Aoyama T, Okamoto Y, Miwa S, et al. Purification of a novel endothelin-converting enzyme specific for big endothelin-3. FEBS Lett. 1998; 428:304–8. [PubMed: 9654154]
9. Hoeper M, Dupuis J. Endothelin receptor antagonists in pulmonary arterial hypertension. Eur Respir J. 2008; 31:407–15. [PubMed: 18238950]
10. Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, et al. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell. 1994; 79:1267–76. [PubMed: 8001159]
11. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA. 1989; 86:2863–7. [PubMed: 2649896]
12. Jasmin JF, Cernacek P, Dupuis J. Activation of the right ventricular endothelin (ET) system in the monocrotaline model of pulmonary hypertension: response to chronic ETA receptor blockade. Clin Sci (Lond). 2003; 105:647–53. [PubMed: 12823096]
13. McCulloch KM, MacLean MR. EndothelinB receptor-mediated contraction of human and rat pulmonary resistance arteries and the effect of pulmonary hypertension on endothelin responses in the rat. J Cardiovasc Pharmacol. 1995; 26(Suppl 3):S169–76. [PubMed: 8587353]
14. Michel RP, Langleben D, Dupuis J. The endothelin system in pulmonary hypertension. Can J Physiol Pharmacol. 2003; 81:542–54. [PubMed: 12839266]
15. Migneault A, Sauvageau S, Villeneuve L, Thorin E, Fournier A, Leblanc N, et al. Chronically elevated endothelin levels reduce pulmonary vascular reactivity to nitric oxide. Am J Respir Crit Care Med. 2005; 171:506–13. [PubMed: 15579730]
16. Miyauchi T, Yorikane R, Sakai S, Sakurai T, Okada M, Nishikibe M, et al. Contribution of endogenous endothelin-1 to the progression of cardiopulmonary alterations in rats with monocrotaline-induced pulmonary hypertension. Circ Res. 1993; 73:887–97. [PubMed: 8403258]
17. Montani D, Souz R, Binkert C, Fischli W, Simonneau G, Clozel M, et al. Endothelin-1/endothelin-3 ratio: a potential prognostic factor of pulmonary arterial hypertension. Chest. 2007; 131:101–8. [PubMed: 17218562]
18. Peacock AJ, Dawes KE, Shock A, Gray AJ, Reeves JT, Laurent GJ. Endothelin-1 and endothelin-3 induce chemotaxis and replication of pulmonary artery fibroblasts. Am J Respir Cell Mol Biol. 1992; 7:492–9. [PubMed: 1419025]
19. Prie S, Stewart DJ, Dupuis J. EndothelinA receptor blockade improves nitric oxide-mediated vasodilation in monocrotaline-induced pulmonary hypertension. Circulation. 1998; 97:2169–74. [PubMed: 9626178]
20. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature. 1990; 348:732–5. [PubMed: 2175397]
21. Sauvageau S, Thorin E, Caron A, Dupuis J. Endothelin-1-induced pulmonary vasoreactivity is regulated by ET(A) and ET(B) receptor interactions. J Vasc Res. 2007; 44:375–81. [PubMed: 17495482]

22. Shi W, Cernacek P, Hu F, Michel RP. Endothelin reactivity and receptor profile of pulmonary vessels in postobstructive pulmonary vasculopathy. Am J Physiol. 1997; 273(6 Pt 2):H2558–64. [PubMed: 9435587]

23. Suzuki N, Matsumoto H, Miyauchi T, Goto K, Masaki T, Tsuda M, et al. Endothelin-3 concentrations in human plasma: the increased concentrations in patients undergoing haemodialysis. Biochem Biophys Res Commun. 1990; 169:809–15. [PubMed: 2192712]

24. Tsai YT, Lin HC, Yang MC, Lee FY, Hou MC, Chen LS, et al. Plasma endothelin levels in patients with cirrhosis and their relationships to the severity of cirrhosis and renal function. J Hepatol. 1995; 23:681–8. [PubMed: 8750167]

25. Wolf SC, Smoltczyk H, Brehm BR, Erley CM, Risler T. Endothelin-1 and endothelin-3 levels in different types of glomerulonephritis. J Cardiovasc Pharmacol. 1998; 31(Suppl 1):S482–5. [PubMed: 9595519]

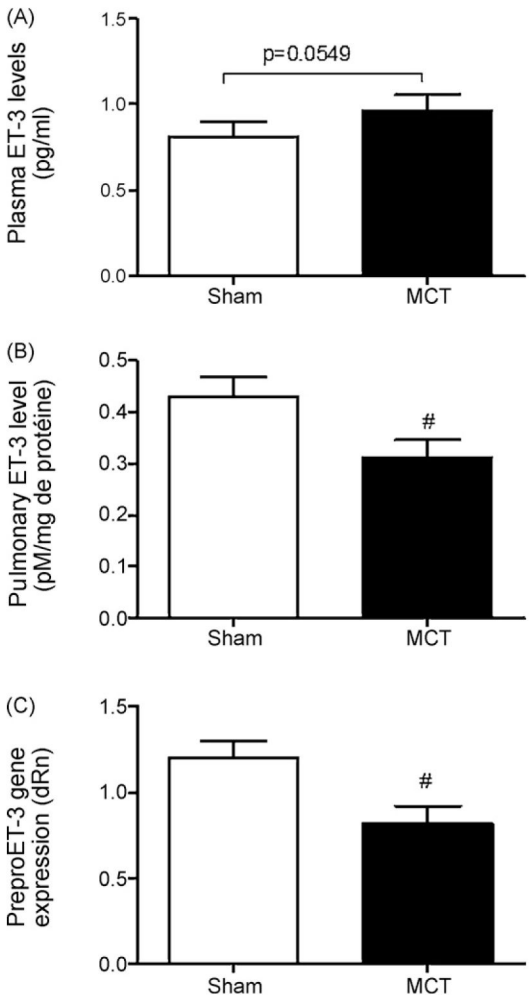

Fig. 1.

ET-3 levels. (A) Arterial plasma ET-3 levels in sham ($n = 19$) and MCT rats ($n = 20$). (B) Pulmonary ET-3 levels in sham ($n = 19$) and MCT rats ($n = 20$). (C) PreproET-3 gene expression in pulmonary resistance arteries normalized with cyclophiline A. Values are expressed as relative quantity (dRn). $^{\#}P < 0.05$.

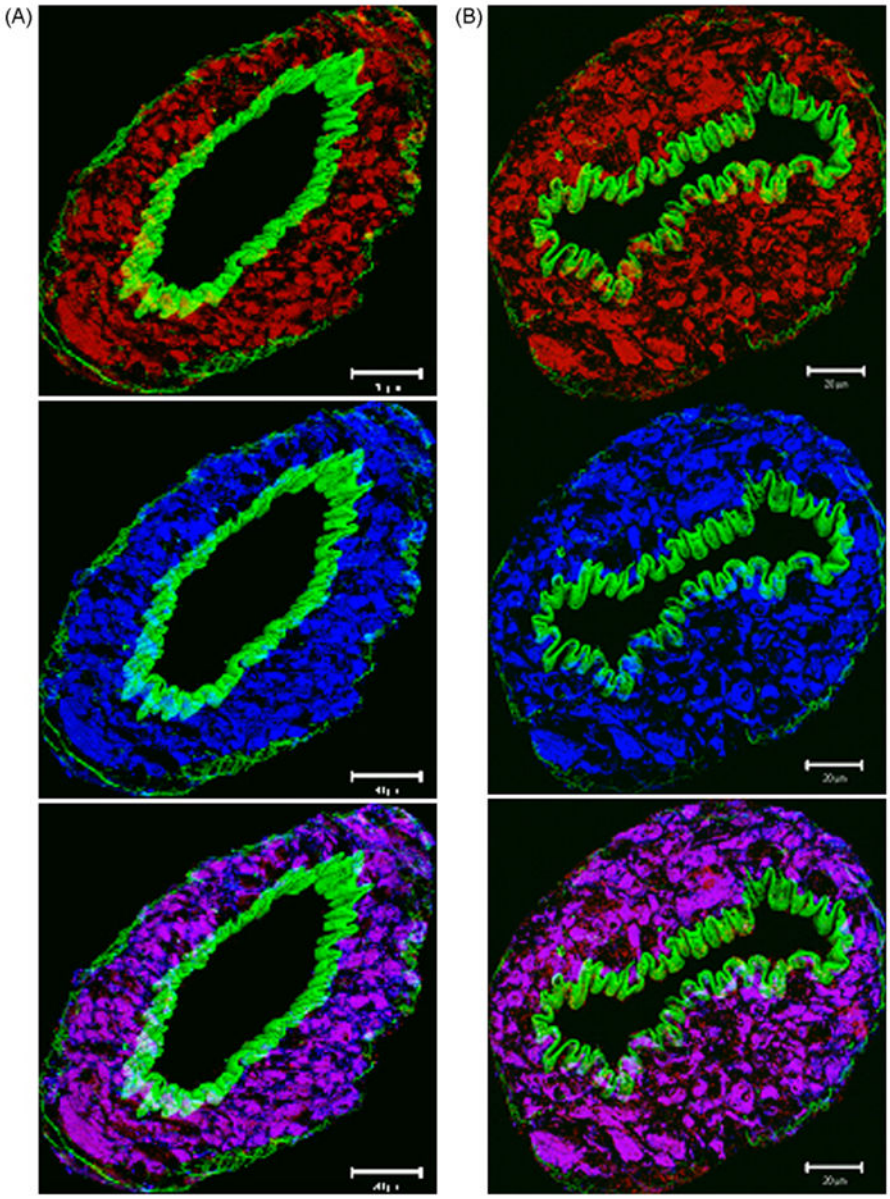

Fig. 2.

Immunofluorescence. Confocal imaging representing the distribution of ET-3 in transverse 8 μmol/l-thick sections of small pulmonary arteries of both sham (A) and MCT rats (B). The images represent examples of composite Z-stacks that were deconvolved and projected with the LSM 510 software. The first row of figures (from left to right) displays the fluorescence of ET-3 (in red) and both the IEL and EEL (in green) which enables easy demarcation of the endothelium from the media. The second row displays the fluorescence of smooth muscle actin (in blue), which is limited to the media. The third row represents the co-localization of ET-3 and smooth muscle actin. The bar/scale represents 20 μm.

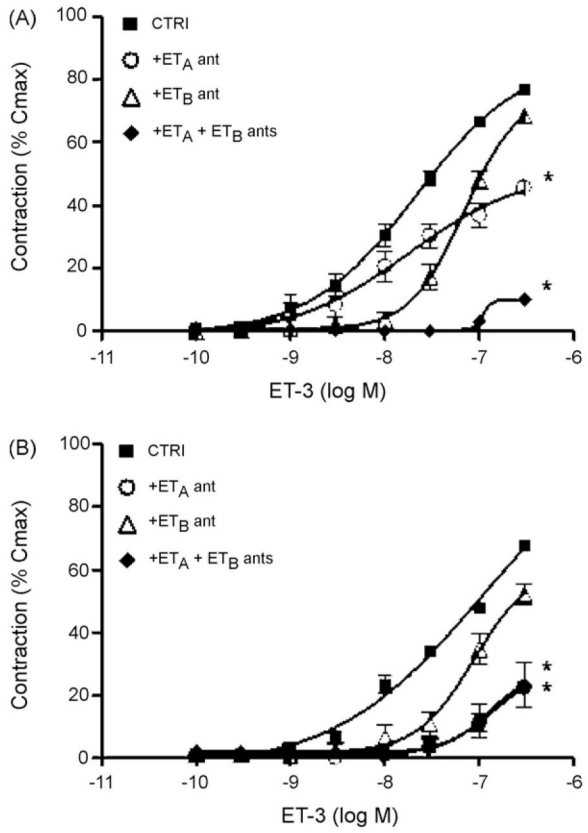

Fig. 3.

Vasoreactivity studies. Endothelin-3-induced pulmonary vasoconstriction in both sham (A) and MCT treated rats (B) in the presence of an ET_A antagonist (open circles, 10 nmol/l) an ETB antagonist (open triangle, 1 μmol/l) and the combination of both (filled diamond); n = 6–8/group. Values are mean ± S.E.M. ET-3-induced vasoconstrictions are expressed as a percentage of the maximal response. $^{*}P < 0.001$.