Deletion of Endothelial Cell Endothelin B Receptors Does Not Affect Blood Pressure or Sensitivity to Salt

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Abstract—Endothelin B receptors in different tissues regulate diverse physiological responses including vasoconstriction, vasodilatation, clearance of endothelin-1, and renal tubular sodium reabsorption. To examine the role of endothelial cell endothelin B receptors in these processes, we generated endothelial cell-specific endothelin B receptor knockout mice using a Cre-loxP approach. We have demonstrated loss of endothelial cell endothelin B receptor expression and function and preservation of nonendothelial endothelin B receptor-mediated responses through binding and functional assays. Ablation of endothelin B receptors exclusively from endothelial cells produces endothelial dysfunction in the absence of hypertension, with evidence of decreased endogenous release of NO and increased plasma endothelin-1. In contrast to models of total endothelin B receptor ablation, the blood pressure response to a high-salt diet is unchanged in endothelial cell–specific endothelin B receptor knockout compared with control floxed mice. These findings suggest that the endothelial cell endothelin B receptor mediates a tonic vasodilator effect and that nonendothelial cell endothelin B receptors are important for the regulation of blood pressure. (Hypertension. 2006;48:286-293.)

Key Words: endothelin • mice • endothelium • receptors, endothelin • vasodilation

Endothelin-1 (ET-1) was initially described as a potent vasoconstrictor and potential mediator of hypertension. More recently, attention has focused on how ET-1 generated within the kidney may promote natriuresis and diuresis and thus contribute to a lowering of blood pressure (BP). ET-1 acts through 2 types of receptors, endothelin receptor type A (ET₁) and endothelin receptor type B (ET₂). Activation of ET₁ and ET₂ on vascular smooth muscle cells results in vasoconstriction. In contrast, vascular endothelial cells (ECs) exclusively express the ET₂ subtype and mediate vasodilatation. ET₂ has been proposed as the target receptor through which collecting duct (CD)–derived ET-1 regulates natriuresis and diuresis. However, ET₂ may also influence BP through regulation of peripheral vascular tone, renal hemodynamics, and clearance of circulating ET-1. The autocrine/paracrine nature of ET-1 signaling, the close interdependence between renal tubular function and intrarenal blood flow, and the modulation of peripheral vascular tone by ET-1 have made the precise mechanisms by which ET₂ on different cell types contribute to the regulation of BP and natriuresis difficult to define, although EC ET₂ may be central to each of these pathways. We hypothesized that CD-derived ET₁ acted in a paracrine manner on medullary vasa recta EC ET₂ to regulate natriuresis and that deletion of these receptors would result in salt-sensitive hypertension. We, thus, adopted a Cre-loxP approach that permitted specific ablation of EC ET₂ while preserving CD ET₂ expression to test this hypothesis.

Methods

The general approach to producing cell type–specific knockout (KO) of ET₂ through Cre-loxP–mediated recombination has been described previously.

Cloning and Mapping of the Mouse ET₂ Receptor Gene

A 129/Ola mouse genomic DNA library was screened using an ET₂ cDNA probe, and a 17.7-kb genomic fragment (clone pAP) was cloned, partially sequenced, and mapped. Sequencing of exons 3, 4, 5, and 6 from pAP showed complete concordance with available published sequences of the ET₂ receptor cDNA.

Construction of Replacement Targeting Vector and Gene Targeting

The replacement vector was based on the Lox²ROSA26 plasmid. A 956-bp region of pAP, spanning exons 3 and 4 of the ET₂ receptor gene, was amplified by PCR (oligo 1: TCCGGATCCAGAGGTCA- TGACCC; oligo 2: TCACTAGTGATGGTTAACCTCACAG) and subcloned into the BamHI site of the vector, such that loxP sites flanked (floxed) the coding regions. A 3.8-kb loxP-flanked MC1: thymidine kinase:phosphoglucokinase:neomycin phosphotransferase (LTLN) selection cassette was then subcloned into the XhoI site 3′ of the PCR product. The 3′ homology arm consisted of the Hpal-Sacl fragment of pAP (exon 5, intron 5, and 56 bp of exon 6), subcloned.
between the XbaI and NolI sites 3' of the selection marker, to produce the plasmid p\(\text{p/H9261P2–6LTNL}\). The 5' homology arm was composed of an 11.9 kb XhoI–NaeI fragment of intron 2. This fragment was subcloned into p\(\text{p/H9261P2–6LTNL}\) upstream of the 5' \(\text{loxP}\) site. The completed construct, p\(\text{p/H9261PW}\) [Figure 1a (i)], thus featured a deletion of 1.1 kb from intron 2 between the NaeI site and a point 288 bp upstream of exon 3. p\(\text{p/H9261PW}\) was linearized by XhoI/NotI digestion and electroporated into E14Tg2a embryonic stem (ES) cells. G418-resistant clones were selected as described15 and DNA extracted for PCR analysis. Two positive controls were run for each DNA sample: oligo 3 (GCCTCTGTTCCACATACACTTCATTC) and oligo 4 (GTAGAAACTGACAGCCACCAATC) amplified a 1.4-kb sequence from within the integrated replacement vector; oligo 5 (TTTGGGTGGTCTCTGTGGTGCTGG) and oligo 6 (GAACAGCTGCCACGTCTC) amplified a 2.4-kb sequence from the wild-type (W) allele; oligo 3 and oligo 6 amplified any specific integration event (\(\text{2.2 kb}\)). Specific recombination at the 3' \(\text{loxP}\) end was confirmed in \(\text{Hind}\III\)-digested DNA with a 1.2-kb external probe complementary to intron 6/exon 7 (Figure 1a and 1b). Cells from targeted clones were electroporated with a Cre recombinase expression plasmid (pMC-Cre16) to remove the selection marker. Gancyclovir-resistant clones were analyzed by PCR. Oligo 5 and oligo 7 (AGCCATAAAGTCACAGCCATTC) amplified a 420-bp sequence spanning the 3' \(\text{loxP}\) site; oligo 8 (TCAGTTGTAATGAGACACAGAC) and oligo 9 (CTTAGAGCACAAAGACTCAGCAC) amplified a 500-bp sequence spanning the 5' \(\text{loxP}\) site. PCR products were sequenced to confirm \(\text{loxP}\) sequences. Targeted ES cell clones were injected into C57BL/6 blastocysts and transferred into C57BL/CBA foster mothers. Chimera were bred to BKW females and progeny genotyped by Southern blot of EcoRI–digested DNA using an internal probe [Figure 1a (iii)] and by PCR using oligos 7 and 8 (Figure 1a and 1c). These PCR oligos flanked an \(\sim2.2\)-kb sequence spanning both \(\text{loxP}\) sites in the targeted (flox) allele and a 2.2-kb sequence in the W allele.

### Generation of EC-Specific ET\(_B\) KO Mice

Homozygous (Flox/Flox) ET\(_B\) mice (background: 50% 129/Ola and 50% BKW) were crossed with Tie2-Cre transgenic mice\(^\ast\) (C57BL/6SJLJ, background). Because of previous reports\(^{18,19}\) of recombination events within germ cells of heterozygous floxed female mice featuring the Tie2-Cre transgene, the transgene was introduced through the male germ line only. Offspring from a single pair in which germ cell recombination was identified were deliberately intercrossed to assess whether a recombination event within the ET\(_B\) gene was sufficient to prevent expression of functional ET\(_B\). Genotyping to identify flox, W, and recombinated alleles was performed by Southern blot and PCR using oligos 7 and 8, as described earlier. The Tie2-Cre transgene was detected by PCR as described.\(^\ast\) All of the mice were given free access to tap water and standard (0.76% NaCl) mouse chow (Special Diet Services, United Kingdom) until implantation of telemeters or in vitro experiments. Mice were housed according to United Kingdom Home

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**Figure 1.** Targeting of the ET\(_B\) receptor gene and genotyping strategy. a, Structure of the targeting vector p\(\text{pPW}\) (i), and ET\(_B\) receptor gene (ii): \(\bullet\) exons; \(\bullet\), \(\text{loxP}\) sites; MC1, Mario Cappeci-1 promoter; TK, thymidine kinase gene; PGK, phosphoglucookinase promoter; Neo, neomycin resistance gene. The positions of internal and external probes used for Southern analysis and of PCR oligonucleotides used for genotyping are illustrated. After homologous recombination events in ES cells, the selection marker was removed by Cre recombinase-mediated excision. The structures of the targeted (Flox) allele (iii) and of the flox allele after in vivo Cre-mediated recombination in ECs (iv) are illustrated. b, Southern blot hybridization analysis with external probe of \(\text{Hind}\III\)-digested genomic DNA from neomycin-resistant targeted (3B3 and 9D3) and nontargeted (2D1 and E14) ES clones. c, PCR analysis (oligos 7 and 8) of genomic tail DNA from W (Ln 1) heterozygous (Ln 2) and homozygous (Flox/Flox) ET\(_B\) receptor mice (Ln 3). In the presence of the Tie2-Cre transgene, heterozygous (Ln 4) and flox/flox (Ln 5) ET\(_B\) receptor mice demonstrate an additional band corresponding to the recombined ET\(_B\) receptor gene allele amplified from tail EC. Ln 6, size marker.
Office recommendations at 22°C with 12-hour diurnal light/dark cycles. All of the procedures were performed under the provisions of the Animals in Scientific Procedures Act (1986) and with the approval of local ethics committees.

**Drugs and Solutions**

ABT627 (ET<sub>A</sub> selective antagonist) and A192621 (ET<sub>B</sub> selective antagonist) were provided by Abbott Pharmaceuticals, United Kingdom. N<sub>ω</sub>-nitro-L-arginine methyl ester hydrochloride (L-NAME), antagonist) were purchased from Calbiochem. Abcam, Germany. Sarafotoxin 6c (S6c; ET<sub>B</sub> selective agonist) was purchased from Calbiochem.

**[125I]-ET-1 Binding by Pulmonary EC**

Lungs were removed and collagenase dispersed as described previously. Protein concentration was measured by the Bradford method, and an aliquot of cells equivalent to 50 μg/mL of membrane protein used for binding reactions. [125I]-ET-1 (7.4 × 10<sup>4</sup> TBq/mmol; American Biosciences, United Kingdom) in HEPES-Ringer/0.2% BSA buffer was added and the volume adjusted to 1 mL with additional buffer. ECs were precipitated with biotinylated Griffonia simplicifolia isolate B4 (GSL I-B4; Vector Laboratories, United Kingdom) immobilized on streptavidin-coated magnetic beads (CELLLection Biotin Binder kit, Dynal Ltd, United Kingdom; 10<sup>5</sup> beads/mL). Radioactivity was measured in a gamma counter and expressed as counts per minute per 50-μg membrane protein. Receptor-specific binding was calculated by subtracting binding in the presence of 3 × 10<sup>-7</sup> M unlabeled ET-1, 3 × 10<sup>-7</sup> M A192621, or 3 × 10<sup>-7</sup> M ABT627 from total [125I]-ET-1 binding.

**Plasma ET-1 Measurement**

Under sodium pentobarbitone anesthesia, 1 mL of blood was withdrawn by direct cardiac puncture in 8- to 12-week-old male mice fed standard chow (0.76% NaCl) from weaning. Plasma ET-1 concentration was measured by radioimmunoassay according to the manufacturer’s instructions (Peninsula Laboratories Europe Ltd, United Kingdom).

**In Vitro Measurement of Vascular and Tracheal Responses**

Isometric contraction and relaxation was measured in 2-mm aortic and tracheal rings. Eight- to 10-week-old male mice were fed standard chow from weaning and culled by CO<sub>2</sub> asphyxiation. Vessels were mounted in myograph chambers and incubated in physiological saline solution (PSS) at 37°C, constantly bubbled with 95%/5% O<sub>2</sub>/CO<sub>2</sub>. A tension of 1.5 g (3.76 mN) for aortic rings and 0.5 g (2.45 mN) for the tracheal rings was applied and vessels equilibrated for 1 hour. Vessels were contracted 3 times with PSS + 125 mmol/L potassium. For aortic rings, a further contraction with 3 × 10<sup>-7</sup> M NE was followed by relaxation with 10<sup>-6</sup> M ACh to assess viability of the endothelium. Vessels that failed to relax by >50% were rejected as evidence of endothelial damage during mounting. Concentration response curves (CRCs) were constructed for NE, NE + 10<sup>-4</sup> M L-NAME, ACh, ACh + 10<sup>-4</sup> M A192621, or vehicle (0.1% DMSO) and for DET-A-NO. Vasodilatation was measured in aortas contracted with NE to 80% of the maximal NE-induced response (NE<sub>max</sub>) and relaxation expressed as percentage of NE<sub>max</sub>. Aortic relaxation to 10<sup>-7</sup> M S6c was measured after 30 minutes of incubation with either 10<sup>-7</sup> M A192621 or vehicle. For tracheas, CRCs were constructed for carbachol (10<sup>-8</sup> M to 3 × 10<sup>-5</sup> M; data not shown) and S6c after incubation with either 10<sup>-7</sup> M A192621 or vehicle. All of the contractile responses were expressed as the percentage of final PSS + 125 mmol/L of potassium-induced contraction.

**Telemetry BP Recording**

Male flox/flox Tie2-Cre and flox/flox litter mate control mice (12 to 16 weeks; 24 to 36g) were housed in single cages for 1 week before surgery and fed from weaning on standard mouse chow prepared as a gel to allow accurate measurement of intake. Under isoflurane anesthesia, a telemetry catheter was inserted into the left carotid artery and the transmitter device (Data Sciences) secured in the left flank as described previously. Mice continued standard gel chow for 7 days after surgery before commencement of high (7.6% NaCl + H<sub>2</sub>O ad libitum), medium (2.5% NaCl + 1% saline ad libitum), or low (0.76% NaCl + H<sub>2</sub>O ad libitum) salt diets. A cohort of mice at the end of the study additionally received A192621 (30 mg/kg per day) in their gel. This dose has been shown previously to inhibit ET<sub>F</sub>-mediated hemodynamic responses in the rat. All of the dietary and drug treatments were maintained for 7 days. Systolic and diastolic BP and heart rate were recorded as described previously and analyzed using the Powerlab data acquisition system. All of the BP results represent the mean of values recorded over the final 24 hours of each dietary/drug intervention.

**Effect of Age on Heart:Body Weight Ratio**

Hearts were dissected from young mice (8 to 12 weeks old) and from older animals (52 to 60 weeks old) fed 0.76% NaCl diet from weaning. The ratio of total ventricular weight (TVW) in milligrams: body weight (BW) in grams was calculated.

**Statistical Analysis**

MAP data were compared by 1-way ANOVA with Newman–Keuls multiple comparison post test analysis. Pulmonary ET<sub>F</sub>-binding and TVW/BW ratio were compared using an unpaired Student t test. CRCs were constructed by linear regression analysis and compared by 2-way ANOVA. EC<sub>50</sub> and E<sub>max</sub> values, S6c-induced vasodilatation, and plasma ET-1 concentrations were each compared by 1-way ANOVA with Dunnett’s multiple comparison post test analysis. Dose ratios were calculated as EC<sub>50</sub> in the presence of antagonist divided by EC<sub>50</sub> in the absence of antagonist. P values were accepted as statistically significant when <0.05. All of the analyses were performed using Graphpad Prism 3.0 software.

**Results**

**Generation of EC-Specific ET<sub>B</sub> KO Mice**

A fragment of the ET<sub>B</sub> receptor gene was replaced with a targeting vector featuring loxp sites flanking exons 3 and 4 and a loxp-flanked selection cassette (Figure 1a). Homologous recombination was detected in 6 of 97 colonies analyzed (Figure 1b). Of these, 3 clones underwent transfection with the targeting vector featuring loxP-flanked selection cassette (Figure 1b). Sequencing confirmed no mutations within loxp sites or coding regions. Germ line transmission was achieved from a single clone and offspring confirmed as heterozygous for the flox allele by PCR (Figure 1c) and Southern analysis. Flox/flox mice were bred by intercross and backcross of heterozygotes. No abnormality of pigmentation, gut development, litter size, or mortality rate was observed in flox/flox mice, and no deviation from Mendelian distribution of alleles was seen in intercross experiments. Flox/flox mice were crossed with Tie2-Cre transgenic mice. A single PCR reaction (Figure 1c) permitted differentiation between W, flox, and recombinated alleles. In flox/W Tie2-Cre mice, a 186-bp band was detected in addition to the flox and W allele, consistent with recombination in a subgroup of cells. Sequencing confirmed the predicted sequence of the recombinated ET<sub>B</sub> allele. Southern analysis, in contrast to PCR, did not detect the recombinated allele, presumably because of the small proportion of tail DNA derived from EC. Male flox/W Tie2-Cre mice were crossed with flox/flox females to produce flox/flox Tie2-Cre mice (EC-specific ET<sub>B</sub> KOs). Again, the recombinated allele
was detected by PCR but not Southern analysis in homozygous mice (Figure 1c).

Generation of Complete ETB KO (Piebald) Mice
We exploited the phenomenon of recombination events in germ cells of female flox/W Tie2-Cre mice to produce offspring homozygous for the recombined allele. Consistent with functional ETB deficiency, these mice exhibited white coat spotting (piebald appearance) because of the regional absence of neural crest–derived melanocytes and died shortly after weaning from intestinal obstruction.25 In contrast to EC-specific KOs, both PCR and Southern analysis detected the recombined allele in piebalds.

ETB-Mediated ET-1 Binding Is Decreased in ECs of EC-Specific ETB KO Mice
ETB-mediated binding of [125I]-ET-1 in EC-enriched populations was decreased by 82% (P<0.001) in flox/flox Tie2-Cre pulmonary cells compared with W controls (Figure 2). ETA-mediated binding did not differ between groups.

ETB-Mediated Vasodilatation Is Impaired in EC-Specific ETB KO Mice
S6c (selective ETB agonist) produced vasodilatation of aortic rings in all genotypes (Figure 3a). The vasodilator response was significantly attenuated in flox/flox Tie2-Cre mice compared with W/W and single transgenic litter mates (P<0.05). Previous incubation with A192621 significantly inhibited S6c-induced vasodilatation in W/W and single transgenic mice (P<0.05 versus no antagonist).

ETB-Mediated Responses in Non-ECs Are Maintained in EC-Specific ETB KO Mice
No abnormality of gut development or pigmentation was observed, consistent with functional ETB expression on neuroblasts and melanoblasts, respectively. S6c-induced tracheal SMC contraction (Figure 3b) was unaltered in EC-specific ETB KO mice. In contrast, tracheas from piebald mice (complete ETB KO) demonstrated no contractile response to S6c. Previous incubation with A192621 significantly attenuated maximal S6c-induced contraction in W/W and flox/flox Tie2-Cre mice (P<0.0001 versus no antagonist).

BP and Heart Rate Are Unaffected by EC-Specific ETB KO
Increasing dietary salt resulted in an increase in BP in both groups of mice (n=6 to 13). However, at no point during each salt treatment was there a difference in systolic, mean, or diastolic BP between control and EC-specific ETB KO mice.

ETB-Mediated Vasodilatation Is Decreased in ECs of EC-Specific ETB KO Mice
Figure 2. EC ETB-mediated binding is decreased in flox/flox Tie2-Cre mice. ETB-dependent binding of [125I]-ET-1 was significantly decreased in EC-enriched pulmonary cells from flox/flox Tie2-Cre mice (P<0.001). Total and ETA-mediated binding did not differ between groups (n=3 in each group).

Figure 3. Selective loss of in vitro EC ETB-mediated vasorelaxation in flox/flox Tie2-Cre mice. a, In vitro vasodilatation to 10^{-7} M S6c was significantly impaired in aortic rings from flox/flox Tie2-Cre mice (P<0.05) and in control animals after selective ETB antagonism (P<0.05 vs no antagonist; n=10 in all groups). b, In vitro S6c-induced ETB-mediated tracheal SMC contraction was normal in flox/flox Tie2-Cre mice (P=0.21 vs W/W; n=16 in each group) but absent in piebald mice (P<0.0001 vs W/W and flox/flox Tie2-Cre; n=10). Previous incubation with A192621 significantly inhibited contractile responses to S6c in flox/flox Tie2-Cre and W/W tracheal rings (Emax 5.36±1.92 [data not illustrated on graph]; P<0.0001 vs no antagonist; n=16 in all groups).
Treatment for 7 days with a selective pharmacological ET\textsubscript{B} antagonist during high-salt diet resulted in further increases in BP (flox/flox 159.7±4.0; \(P<0.05\) versus 7.6% NaCl; \(n=7\)), although the increase just failed to reach statistical significance in flox/flox Tie2-Cre mice (154±2.9; \(P=0.06\) versus 7.6% NaCl; \(n=6\); Figure 5). Heart rate (≈570 bpm) was not influenced by salt intake, genotype, or pharmacological ET\textsubscript{B} antagonism (data not shown).

**Aged EC-Specific ET\textsubscript{B} KO Mice Do Not Develop Cardiac Hypertrophy on Normal Salt**

TVW/BW ratio (mg/g) did not differ between control and EC-specific KO mice in either age group, consistent with the lack of BP difference observed during telemetric studies (young mice flox/flox 3.56±0.13, flox/flox Tie2-Cre 3.74±0.22; \(P=0.48\); \(n=12\) to 13; aged mice flox/flox 4.13±0.29, flox/flox Tie2-Cre 4.59±0.32; \(P=0.31\); \(n=8\) to 9).

**Impaired ACh-Induced Vasodilatation in Aortas From EC-Specific ET\textsubscript{B} KO Mice and W Aortas Pretreated With ET\textsubscript{B} Antagonist**

EC-specific ET\textsubscript{B} KO resulted in significantly impaired ACh-induced vasodilatation (Figure 6a). Previous incubation with A192621 for 7 days. Values are MAP during the final 24 hours of dietary or drug treatment (\(n=6\) to 13 per group). There were no differences in BP between genotypes during any of the dietary/drug treatments.

**Discussion**

We describe a novel transgenic line in which ET\textsubscript{B} expression is regulated by cell type–specific production of Cre recombinase. We have demonstrated that the floxed ET\textsubscript{B} receptor gene functions normally but that expression of Cre recombinase in flox/flox ET\textsubscript{B} mice effectively inactivates ET\textsubscript{B} expression from both alleles. EC-specific inactivation of ET\textsubscript{B} results in increased plasma ET-1 concentration and impaired endothelium-dependent vasodilatation but not hypertension. In contrast to models of rescued complete ET\textsubscript{B} KO\textsuperscript{8,26} and CD-specific ET-1 KO,\textsuperscript{3} EC-specific KO mice do not demonstrate increased sensitivity to high-salt diets. This suggests that the likely target(s) for CD-derived ET-1 in the kidney that determine the salt-sensitive phenotype are ET\textsubscript{B} located on non-EC, most likely those on inner medullary CD cells.\textsuperscript{27} Consistent with previous reports,\textsuperscript{26} the salt-sensitive phenotype seems to be independent of endothelial dysfunction resulting from ET\textsubscript{B} deficiency.

We have demonstrated that loss of ET\textsubscript{B} does not influence BP, regardless of dietary salt intake. After a high-salt diet, rescued ET\textsubscript{B}-deficient rats\textsuperscript{8} and mice\textsuperscript{26} and ET\textsubscript{B} antagonist–treated rats\textsuperscript{8} develop grossly elevated BP compared with controls. We observed a clear relationship between salt intake and BP in our mice, with significant increases in BP observed when dietary NaCl intake exceeded \(\approx 2.5\%\). Modest hypertension during salt loading has been described in several mouse strains, particularly those featuring 2 copies of the renin gene. The genetic background of our mice features DNA derived from 129/Ola strain (2 renin genes\textsuperscript{28}) and C57BL/6, a single renin gene strain that can also develop increased BP after salt loading.\textsuperscript{24,29} Although the influence of salt in our genetic background may have obscured subtle changes in BP secondary to ET\textsubscript{B} KO, we still found that selective pharmacological antagonism of ET\textsubscript{B} during high-salt diet led to further increases in BP, as described previously in the rat.\textsuperscript{9} Although there was no difference in BP between control and KOs during pharmacological ET\textsubscript{B} blockade, the absolute increase in BP just failed to reach statistical significance in EC-specific KOs (\(P=0.06\)). The potential effects of ET\textsubscript{B} KO on a salt-resistant genetic background are, thus, intriguing and merit further study.

We report that loss of endogenous ET\textsubscript{B} activity, through either EC-specific ET\textsubscript{B} KO or acute exposure to ET\textsubscript{B}-selective antagonists, results in endothelial dysfunction, as assessed by ACh-induced vasodilatation. The lack of effect on vasodilatation of A192621 in EC-specific ET\textsubscript{B} KOs suggests that this is predominantly because of loss of EC ET\textsubscript{B} function and is likely related to decreased NO bioavailability, because relaxation to ACh is mainly mediated by NO in the mouse aorta.\textsuperscript{30,31} Diminished NO bioavailability may result from loss of endogenous EC ET\textsubscript{B}–mediated NO release, as suggested by the acute effect of pharmacological ET\textsubscript{B} inhibition in W vessels. In addition, the modest enhancement of NE-induced vasoconstriction after
L-NAME indicates that vascular tone in EC-specific ET_{B} KO mice is less dependent on endogenous NO release than in W mice. However, NO bioavailability may also be influenced indirectly by increased plasma ET-1. EC-specific overexpression of a prepro-ET-1 transgene increases reduced nicotinamide-adenine dinucleotide phosphate oxidase activity, superoxide production, and vascular remodeling but does not increase BP. Recent studies have identified that primary abnormalities of vascular smooth muscle cell tone in either peripheral resistance vessels or the renal vasculature may directly cause hypertension. This challenges the established hypothesis that primary abnormalities of renal sodium excretion are required for hypertension to develop. We have found that deletion of EC ET_{B} from peripheral resistance vessels and from the renal vasculature does not alter BP, despite endothelial dysfunction and an increase in plasma ET-1 concentration. However, prolonged exposure to increased ET-1 may have caused downregulation or desensitization of ETA, decreasing both vascular tone and BP in EC ET_{B} KO mice. We analyzed ETA expression by quantitative autoradiography and assessed functional responses to infusion of big ET-1 (1 to 10 nmol/kg) during anesthesia. ETA expression was unchanged in EC ET_{B} KO mice, and the increase in BP observed after incremental doses of big ET-1 did not differ from that of control mice (data not shown). Thus, we consider it unlikely that downregulation or desensitization of vascular vasoconstrictor ETA activity obscured any hypertensive phenotype in EC ET_{B} KO mice.

Recent studies have identified that primary abnormalities of vascular smooth muscle cell tone in either peripheral resistance vessels or the renal vasculature may directly cause hypertension. This challenges the established hypothesis that primary abnormalities of renal sodium excretion are required for hypertension to develop. We have found that deletion of EC ET_{B} from peripheral resistance vessels and from the renal vasculature does not alter BP, despite endothelial dysfunction and an increase in plasma ET-1 concentration. However, prolonged exposure to increased ET-1 may have caused downregulation or desensitization of ETA, decreasing both vascular tone and BP in EC ET_{B} KO mice. We analyzed ETA expression by quantitative autoradiography and assessed functional responses to infusion of big ET-1 (1 to 10 nmol/kg) during anesthesia. ETA expression was unchanged in EC ET_{B} KO mice, and the increase in BP observed after incremental doses of big ET-1 did not differ from that of control mice (data not shown). Thus, we consider it unlikely that downregulation or desensitization of vascular vasoconstrictor ETA activity obscured any hypertensive phenotype in EC ET_{B} KO mice.

Two hypotheses may explain why EC-specific ET_{B} KO mice, in contrast to mice with complete ET_{B} deficiency, do not develop severe salt-sensitive hypertension. First, the ET-1/ET_{B} signaling pathway regulating tubular reabsorption of sodium and water

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**Figure 6.** EC-specific ET_{B} KO results in endothelial dysfunction and impaired NO release. a, ACh-induced vasodilatation was significantly impaired in flox/flox Tie2-Cre mice (logEC_{50} flox/flox Tie2-Cre 6.30 ± 0.04; W/W 6.76 ± 0.06; P<0.001; E_{max} flox/flox Tie2-Cre 76.84 ± 2.86; W/W 90.30 ± 3.05; P<0.05). Previous incubation with A192621 significantly impaired ACh-induced vasodilatation in W but not flox/flox Tie2-Cre mice (logEC_{50} flox/flox Tie2-Cre +A192621 6.33 ± 0.06; P>0.05 vs no antagonist; W/W +A192621 6.38 ± 0.08; P<0.001 vs no antagonist; E_{max} flox/flox Tie2-Cre +A192621 72.86 ± 2.50; P>0.05 vs no antagonist; W/W +A192621 73.11 ± 3.22; P<0.001 vs no antagonist; n=6 in all groups). b, Endothelium-independent vasodilatation to DETA-NO did not differ between W and flox/flox Tie2-Cre aortic rings (logEC_{50}; flox/flox Tie2-Cre 5.46 ± 0.09; W/W 5.51 ± 0.07; P>0.05; E_{max} flox/flox Tie2-Cre 87.48 ± 4.82; W/W 85.52 ± 3.38; n=6). c and d, NE-induced contraction was significantly enhanced in both W/W (P<0.0001) and flox/flox Tie2-Cre (P<0.0001) mice after NOS inhibition (logEC_{50}; W/W 7.15 ± 0.15; W/W +L-NAME 8.15 ± 0.06; flox/flox Tie2-Cre 7.47 ± 0.06; flox/flox Tie2-Cre +L-NAME 7.87 ± 0.07; n=6). However, the magnitude of the increased contractile response after L-NAME was significantly greater in W than in EC-specific KOs.
may be an autocrine process mediated solely by inner medullary CD cells27 and, as such, would be unaffected by loss of EC ETα. Alternatively, the effect of loss of any paracrine ET-1/ETα signaling pathway involving CD cells and medullary vasa recta EC13 that might normally regulate natriuresis by alteration of medullary blood flow11 may have been masked by changes in the glomerular filtration rate. Both ETα and ETβ regulate afferent arteriolar vasoconstriction, whereas EC ETα produces vasodilation of efferent arterioles.44 The balance between ET-1-mediated vasoconstriction and vasodilation in the glomerulus may, thus, be important in determining tubular sodium delivery. Key targets for future studies will be to examine the effects of selective inner medullary CD ETα KO on sodium and water handling and to determine the influence of vascular ETβ on glomerular function and medullary blood flow.

Perspectives

This article describes a novel murine model of ETβ deficiency that permits investigation of the physiological influence of ETβ expressed on a single cell type, without the confounding influence of loss of ETβ from other cell types. This model will be useful for in vivo studies of ET-1/ETβ physiology, particularly in organs such as the kidney, where tubular and vascular functions regulated by ET-1 are closely interlinked, making physiological studies using selective antagonists difficult to interpret.

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Disclosures

None.

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