Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention

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Ge, Yuqiang, Alan Bagnall, Peter K. Stricklett, Kevin Strait, David J. Webb, Yuri Kotelevtsev, and Donald E. Kohan. Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. Am J Physiol Renal Physiol 291: F1274–F1280, 2006.—Collecting duct (CD)-derived endothelin-1 (ET-1) inhibits renal Na reabsorption and its deficiency increases blood pressure (BP). The role of CD endothelin B (ETB) receptors in mediating these effects is unknown. CD-specific knockout of the ETB receptor was achieved using an aquaporin-2 promoter-Cre recombinase transgene and the loxP-flanked ETB receptor gene (CD ETB KO). Systolic BP in mice with CD-specific knockout of the ETB receptor, ETA receptor (CD ETA KO) and ET-1 (CD ET-1 KO), and their respective controls were compared during normal- and high-sodium diet. On a normal-sodium diet, CD ETB KO mice had elevated BP, which increased further during high salt feeding. However, the degree of hypertension in CD ETB KO mice and the further increase in BP during salt feeding were lower than that of CD ET-1 KO mice, whereas CD ETA KO mice were normotensive. CD ETB KO mice had impaired sodium excretion following acute sodium loading. Aldosterone and plasma renin activity were decreased in CD ETB KO mice on normal- and high-sodium diets, while plasma and urinary ET-1 levels did not differ from controls. In conclusion, the CD ETB receptor partially mediates the antihypertensive and natriuretic effects of ET-1. CD ETA and ETB receptors do not fully account for the antihypertensive and natriuretic effects of CD-derived ET-1, suggesting paracrine effects of this peptide.

Numerous lines of evidence indicate that collecting duct (CD)-derived endothelin-1 (ET-1) is an important regulator of renal Na reabsorption and systemic blood pressure (BP). First, the CD is the major renal site of ET-1 production (8, 25, 41, 48, 49), synthesizing more of the peptide than any other cell type (24). Second, the distribution of ET receptors in the kidney closely corresponds to the sites of ET production. Binding and RT-PCR studies using microdissected nephron segments indicate that endothelin receptors are primarily expressed by the inner medullary CD, moderately expressed by outer medullary CD and cortical CD, with much lower expression by other nephron segments (43, 45). Third, cultured inner medullary CD cells secrete ET-1 from, and bind ET-1 to, the same (basolateral) side (27). Fourth, in vitro studies indicate that ET-1 inhibits CD Na transport. ET-1 decreases Na reabsorption by the isolated CD, an effect that may be mediated by inhibition of amiloride-sensitive sodium channel activity (14, 31, 32, 46) and/or Na-K-ATPase activity (53). Perhaps the most compelling evidence implicating CD-derived ET-1 in the regulation of renal Na excretion and BP comes from gene targeting studies. Mice with CD-specific knockout of the ET-1 gene (CD ET-1 KO) are hypertensive on a normal-Na diet and this is exacerbated by high Na intake, with systolic BP increasing by almost 40 mmHg compared with controls (1). CD ET-1 KO mice also have impaired ability to excrete a Na load which is partly restored by inhibition of the CD epithelial Na channel with amiloride (1). Taken together, the above data indicate that CD-derived ET-1 is an important physiological regulator of systemic BP and that this effect is at least partially mediated through autocrine inhibition of CD Na reabsorption.

An important question that arises from the above studies is which ET receptor(s), if any, in the CD mediates the strong antihypertensive effect of CD-derived ET-1. Two major ET receptor subtypes have been identified, ETA and ETB. Numerous studies show that CD express ETB receptors (10, 23, 26, 43, 45). ET-1 reduces vasopressin-stimulated cAMP accumulation (29, 47) and osmotic water permeability (33, 36) in the CD, an effect that, at least in vitro, is mediated by activation of the ETB receptor (12, 29, 43, 46). In A6 cells, a distal nephron cell line, stimulation of the ETB receptor inhibits apical Na entry (14). Whole animal knockout of the ETB receptor is lethal shortly after birth due to aganglionic megacolon (21); however, these animals have been rescued by transgenic gut-specific expression of the ETB receptor (16). Rescued ETB-deficient rats have marked salt-sensitive hypertension that is partially normalized by amiloride, suggesting that the ETB receptor, by regulating the CD apical Na channel, is responsible for maintaining BP homeostasis (15). However, this same group reported that when normal kidneys were transplanted into ETB-deficient rats, BP remained elevated and sodium excretion was still impaired (35). Thus the salt-sensitive hypertension in genetically ETB receptor-deficient rats could not be ascribed wholly to renal ETB receptor absence. Hence, while in vitro data are suggestive, there is no firm evidence yet that the CD ETB receptor does, in fact, mediate the natriuretic and antihypertensive effects of CD-derived ET-1.

In contrast to ETB receptors, initial studies yielded controversial data on whether CD express ETA receptors. Some investigators detected only ETB receptors in the CD (10, 22, 43, 45), while others, using immunohistochemistry, binding analysis, or RT-PCR, found ETA receptor expression in CD (7, 24).
12, 26, 37, 50, 51). The most definitive evidence for CD ETA receptor expression comes from studies in which the ETA receptor was specifically targeted in this nephron segment (CD ETA KO). CD ETA KO mice had reduced vasopressin-induced cAMP accumulation, providing evidence for CD ETA receptor expression and functionality (18). However, these mice did not have altered systemic BP or renal Na excretion, suggesting that, at least under physiological conditions, the CD ETA receptor is not involved in BP regulation.

Taken together, the above studies clearly implicate CD ET-1 as an important physiological regulator of renal Na excretion and BP. However, some fundamental questions remain. It is unclear whether CD-derived ET-1 exerts these effects primarily through activation of CD ET receptors in an autocrine fashion, or whether significant paracrine interaction with adjacent cell types occurs. If the latter scenario were true, it would represent one of the first reports of a tubule-derived factor having a physiologically relevant paracrine effect. Another key question is which ET receptor subtype(s) in the CD, if any, are involved in mediating the ET-1 effect. Such information would have substantial relevance to understanding the fluid retention and edema formation that occur with administration of ET receptor antagonists to patients (3). While CD ET-1 and ETA receptor knockouts have provided much information on this system, a key missing piece is the role of the CD ETB receptor. Such information would provide important answers to the above questions; consequently, the current study was undertaken to determine the effect of selective disruption of ETB receptors in the CD on BP and renal Na excretion.

MATERIALS AND METHODS

Transgenic mice lines. Mice with CD-specific disruption of the ETB receptor gene were generated in a manner similar to that previously described for CD-specific ET-1 KO (1). ETB construct design, gene targeting, and generation of all studies. Amplification for the AQP2-Cre transgene using oligonucleotide primers mAQP2-Cre (CD ETB KO) were used in all studies. Stem Cell Research, University of Edinburgh by AB and YK. Floxed ETB receptor gene were generated in a manner similar to that above questions; consequently, the current study was undertaken to determine the effect of selective disruption of ETB receptors in the CD on BP and renal Na excretion.

Genotyping. Tail DNA was prepared by standard methods and PCR amplified for the AQP2-Cre transgene using oligonucleotide primers mAQP2-Cre (located in intron 2 in the sequence that was deleted when constructing the 5′ loxP site), and ETBF1 5′-AGCCATAAAGTCCAGGCA-3′ (located in intron 4, 3′ to the second loxP site). Products of primers ETBF1 and ETBR3 give a product only in wild-type animals (865 bp), while ETBF1 and ETBR1 give products of 1,092 bp in unrecombined DNA and 186 bp after recombination. PCR products were visualized after electrophoresis through 1.5% agarose.

Chronic telemetry and metabolic balance studies. All mice were studied around 3 mo of age. All mice had catheters inserted into the right carotid artery, tunneled subcutaneously, and the attached radio-transmitter localized to the back. Continuous recording of BP and pulse was performed using telemetry (Data Sciences International, Arden Hills, MN). Two days after the surgery, mice were placed into Nalgene metabolic cages (Rochester, NY) and acclimated for 3 days. Mice were fed 6 ml of a gelled diet that contained all nutrients and water as previously described (1). Hemodynamic values were not recorded during this conditioning period. After the 3-day acclimation period, metabolic balance studies were performed for 3 consecutive days. Daily gel intake and body weights were measured and urine collected under oil. Systolic, diastolic, and mean BPs, as well as pulse rate, were averaged over the course of each day. At the end of this baseline period, ~100 μl of blood were taken from the tail vein for determination of plasma renin activity (PRA) and serum electrolytes (Na and K). In some studies, mice were killed and blood obtained by cardiac puncture for determination of plasma ET-1 and creatinine concentrations. The urine from each day was analyzed for volume, Na and K concentration, while urine from the third day of baseline studies was also used for determination of aldosterone, ET-1, and creatinine excretion rates.

For Na-loading studies, mice were examined as described above except that after 3 days of a normal (0.3%)-Na diet, mice were placed on a high-Na diet for 7 days. The high-Na diet consisted of 6 ml of gelled diet containing 1% Na plus normal saline to drink. Daily weights and telemetry were obtained, and urine collected for determination of Na and K concentration. At the end of the 7-day period, mice were killed and bled for determination of PRA, plasma ET-1, Na, K and creatinine concentrations. Urine from day 7 of the high-Na diet was also used for determination of aldosterone, ET-1, and creatinine excretion rates.

Mice with CD-specific knockout of ET-1 (CD ET-1 KO) or the ETA receptor (CD ETA KO) were also utilized to compare systolic BPs obtained by telemetry and by tail cuff plethysmography (latter measures only systolic BP). Tail cuff plethysmography was performed using the previously described system (1) (BP-2000, Visitech Systems, Apex, NC). The CD ET-1 KO and CD ETA KO mice have been previously described in detail (1, 17, 18).

Acute Na loading. After fasting for 12 h, mice were placed into small metabolic cages that contained no food or water and given 1.5 ml normal saline intraperitoneally. Subsequently, urine was collected under oil on an hourly basis for the next 6 h. Urine was analyzed for volume, Na and K. To control for the volume load, mice were also given 2 ml water ip and urine osmolality and volume were determined for the next 6 h.

ETB receptor gene recombination in nephron segments. Kidney sections were incubated in 1 mg/ml collagenase and 0.1% DNAse at 37°C for 1 h. Proximal tubule, thick ascending limb, and cortical, outer medullary and inner medullary CDs were microdissected, and DNA was isolated. Samples were amplified using real-time PCR (Smart Cycler, Cephid, Sunnyvale, CA). The same primers as used for genotyping were employed.

Electrolyte and hormone analysis. Plasma and urine were analyzed for Na and K concentration (EasyVet analyzer, Medica, Bedford, MA) and creatinine (Jaffe colorimetry, Sigma, St. Louis, MO). PRA was measured as previously described (1) using an indirect radioimmunoassay (Peninsula Laboratories, San Carlos, CA). Aldosterone levels...
were determined by radioimmunoassay, after HCl hydrolysis and ethyl acetate extraction (Coat-a-Count, Diagnostic Products, Los Angeles, CA). Plasma and urine ET-1 were extracted as previously described (1) and analyzed by radioimmunoassay (Peninsula Laboratories).

Statistics and ethics. Comparisons of single points on a single day between control and CD ETB KO mice were analyzed by the unpaired Student’s t-test. Comparisons of multiple points (e.g., BP and Na excretion) were made using one-way ANOVA with the Bonferroni correction. P < 0.05 was taken as significant. Data are expressed as means ± SE.

All animal experiments were ethically approved by the University of Utah Institutional Animal Care and Use Committee.

RESULTS

Characterization of CD ETB KO mice. CD ETB KO mice developed normally until at least 6 mo of age and had no gross morphological abnormalities. As previously described (1), AQP2-Cre mice confer CD-specific knockout, as determined by principal cell-specific Cre recombinase activity in mice heterozygous for AQP2-Cre and the ROSA26-YFP reporter, in situ hybridization, genomic PCR of microdissected CD, and genomic PCR for gene recombination in an organ panel of 15 different organs. This was further confirmed by RT-PCR of ETB receptor mRNA as well as PCR of genomic DNA in microdissected CD. Cortical, medullary, and inner medullary CDs all showed ETB receptor gene recombination, while microdissected proximal tubules and thick ascending limbs had no recombination. In addition, CD from CD ETB KO mice had markedly reduced ETB receptor mRNA (25% of the levels expressed in control CD, n = 15 tubules). It should be noted that microdissected cortical RIH-YFP reporter, in situ hybridization, genomic PCR of microdissected CD, and genomic PCR for gene recombination in an organ panel of 15 different organs. This was further confirmed by RT-PCR of ETB receptor mRNA as well as PCR of genomic DNA in microdissected CD. Cortical, medullary, and inner medullary CDs all showed ETB receptor gene recombination, while microdissected proximal tubules and thick ascending limbs had no recombination. In addition, CD from CD ETB KO mice had markedly reduced ETB receptor mRNA (25% of the levels expressed in control CD, n = 15 tubules). It should be noted that microdissected cortical CD contain intercalated cells which may express ETB receptors and are not targeted by AQP2-Cre. Thus CD ETB KO mice have principal cell-specific inactivation of the ETB receptor gene.

Renal function and BP during a normal-Na diet. As previously described (1), all mice were ration fed in order that food and water intake were exactly matched. This was achieved by using a gelled diet that contained all food and water and that met all nutritional needs. Mice eat all of the provided gel (6 ml gel containing 4.3 ml water) and do not drop any of it into the bottom of the cage due to its stickiness. Under baseline conditions of 0.3% Na intake, CD ETB KO mice had elevated systolic (12.9 ± 2.2 mmHg greater than controls, n = 12, P < 0.01) and diastolic BP (8.2 ± 1.9 mmHg greater than controls, n = 12, P < 0.01; Table 1 and Fig. 1). Plasma ET-1 concentration and urinary ET-1 excretion did not differ between CD ETB KO and control mice after 3 days on a normal-Na diet. In contrast, PRA and urinary aldosterone excretion were significantly lower in CD ETB KO mice compared with controls (see Table 3).

Renal function and BP during a high-Na diet. Following the 3 days of a normal-Na diet, mice were placed on a high-Na diet containing 6 ml gelled 1% Na diet and normal saline to drink. This diet caused an eight- to ninefold increase in urinary Na excretion (Tables 1 and 2 and Fig. 2). CD ETB KO mice had modest, albeit significant, further increases in systolic and diastolic BPs of ~7 and 5 mmHg, respectively (Tables 1 and 2 and Fig. 2). In contrast, BP was unchanged in control mice during a high-Na diet. Despite the increase in BP, no difference in urinary Na excretion could be detected between CD ETB...
Table 2. Metabolic balance data in control and CD ETB KO mice on day 2 of a high-sodium diet

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>Control</th>
<th>CD ETB KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>118.6±2.0</td>
<td>138.9±2.9*</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>97.2±2.1</td>
<td>109.7±2.8*</td>
</tr>
<tr>
<td>Pulse, beats/min</td>
<td>597±26</td>
<td>638±27</td>
</tr>
<tr>
<td>Gel intake, g/day</td>
<td>5.5±0.3</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>Saline intake, ml/day</td>
<td>6.2±1.1</td>
<td>6.1±1.5</td>
</tr>
<tr>
<td>Weight, g</td>
<td>27.1±1.1</td>
<td>27.5±0.9</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>3.60±0.90</td>
<td>3.79±1.11</td>
</tr>
<tr>
<td>[Na] urine, meq/l</td>
<td>653±77</td>
<td>628±81</td>
</tr>
<tr>
<td>[Na] plasma, meq/l</td>
<td>148±1</td>
<td>146±1</td>
</tr>
<tr>
<td>[K] urine, meq/l</td>
<td>79.2±14.1</td>
<td>86.0±13.8</td>
</tr>
<tr>
<td>[K] plasma, meq/l</td>
<td>4.1±0.1</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>Urine K excretion, μeq/day</td>
<td>253±31</td>
<td>274±34</td>
</tr>
<tr>
<td>[Creatinine] urine, mg/dl</td>
<td>14.9±2.8</td>
<td>15.2±2.9</td>
</tr>
<tr>
<td>[Creatinine] plasma, mg/dl</td>
<td>0.24±0.01</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Clcr, ml/min</td>
<td>0.15±0.03</td>
<td>0.16±0.04</td>
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</table>

Values are means ± SE; n = 6–12 each data point. *P < 0.01 vs. control.

KO and control mice on any of the 7 days of high Na intake. Notably, Na intakes and daily weights were similar between the two groups of mice over the course of the high-Na diet. In addition, there were no differences in pulse, fluid intake, urine volume, urine Na or K concentration, plasma Na or K concentration, K excretion or creatinine clearance between CD ETB KO and control animals (Table 2). A high-Na diet did not affect plasma ET-1 concentration, but did increase urinary ET-1 excretion about threefold in both groups of mice, albeit to a similar degree (Table 3). Urinary aldosterone excretion markedly decreased on a high-Na diet in both groups (Table 3). Although the amounts were not different between the ETB KO and control mice, the levels were at the lower limit of detection of the assay so it was not possible to determine if there was a true difference in urinary aldosterone excretion. PRA also fell on a high-Na diet; however, CD ETB KO still had lower levels compared with controls (Table 3).

Effect of acute Na loading. Although CD ETB KO mice had salt-sensitive hypertension, no difference in urinary Na excretion could be detected. However, during chronic Na loading studies, renal function is only measured at 24-h intervals and not until 24 h after Na loading. It was possible, therefore, that short-term differences in renal Na handling might have been overlooked. To test this, mice were given 1.5 ml normal saline intraperitoneally, followed by hourly urine collections for 6 h. As shown in Fig. 3, CD ETB KO mice had substantially reduced urinary Na excretion within the first 2 h after an acute Na load. This decrease was also associated with a decrease in urine volume (0.75±0.11 ml in CD ETB KO and 1.07±0.11 ml in control, n = 6 each group, P < 0.025). Over the next 2-h period, CD ETB KO mice had modestly increased Na excretion compared with controls, indicating that they partially, although not completely, normalized net Na excretion. No difference in Na excretion was detected at 6 h, so after the entire 6-h period ETB KO mice had excreted less Na than controls. To control for a volume load effect, urine concentration was examined for 6 h after a 2-ml acute water load ip. Urine osmolality did not differ between CD ETB KO and control mice (n = 6 each group) after 2 h (224±3 mosmol/kgH2O in CD ETB KO and 273±28 mosmol/kgH2O in control) or after 6 h (1,446±329 mosmol/kgH2O in CD ETB KO and 1,555±419 mosmol/kgH2O in control). Similarly, urine volume was not different after 2 h (0.64±0.08 ml in CD ETB KO and 0.59±0.06 ml in control) or 6 h cumulatively (1.06±0.09 ml in CD ETB KO and 1.10±0.03 ml in control).

Comparison of BP between CD ET-1 knockout mice and CD ET receptor knockout mice. Previous studies from our group indicated that CD ET-1 KO mice were hypertensive on a normal-Na diet and had marked salt-sensitive hypertension associated with frank Na retention over the first 2 days of a high-Na diet (1). However, BP in these studies were measured by tail cuff plethysmography and might not, therefore, be strictly comparable to the telemetric studies in the current work. To address this, CD ET-1 KO mice (n = 6) had BP determined using telemetry while on a normal- and high-Na diet identical to that used for the CD ETB KO studies. These

Table 3. Plasma and urine hormone levels in control and CD ETB KO mice under varying dietary conditions

<table>
<thead>
<tr>
<th>Dietary Condition</th>
<th>Aldosterone, pg ANG I μl−1 h−1</th>
<th>PRA, pg/ml</th>
<th>Plasma ET-1, pg/ml</th>
<th>Urinary ET-1, pg/day</th>
</tr>
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<tbody>
<tr>
<td>Normal Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.53±0.31</td>
<td>4.76±1.12</td>
<td>2.5±0.1</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>CD ETB KO</td>
<td>1.16±0.03*</td>
<td>1.22±0.39*</td>
<td>2.7±0.2</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>High Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.25±0.03</td>
<td>1.75±0.27</td>
<td>2.1±0.2</td>
<td>4.3±1.1</td>
</tr>
<tr>
<td>CD ETB KO</td>
<td>0.26±0.02</td>
<td>0.59±0.25*</td>
<td>2.2±0.2</td>
<td>4.4±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 each data point. Aldosterone, urinary aldosterone excretion; PRA, plasma renin activity. *P < 0.01 vs. control for same dietary condition.
studies were done in the same litter of mice as had tail cuff plethysmography, although tail cuff and telemetry measurements were not made simultaneously in the same mice. Tail cuff measurements were taken over 1 h in the afternoon for 3 consecutive days, while telemetry measurements reflect values averaged for the entire 24-h period over 3 consecutive days. We found that systolic BP was not significantly different whether measured by tail cuff or telemetry. In particular, the difference in systolic BP between CD ET-1 KO and control mice when determined by telemetry did not vary from that determined by tail cuff by more than 10%. Similar studies were performed in CD ETA KO mice (n = 3–4) who once again were found to be normotensive on a normal- or high-Na diet (18). Since the tail cuff data were similar to the telemetry data, and since these tail cuff conditions were used in the original description of the CD ET-1 KO mice, it seems valid to compare the data on systolic BP between CD ET-1 KO and CD ETA KO mice using tail cuff and telemetry measurements. A comparison of systolic BP between mice with CD-specific knockout of ET-1, the ETA receptor, and the ETB receptor knockout is shown in Fig. 4. Data are expressed as the difference in systolic BP between knockout mice and their sex-matched cre-negative littermate control. CD ET-1 KO mice had significantly greater systolic BP during normal salt intake, as well as a greater increase after a high-Na diet, compared with CD ETA KO mice. Since CD ETA KO does not affect BP, these data indicate that the hypertensive effect of CD ET-1 KO can be partially, but not completely, ascribed to lack of activation of the CD ETB receptor.

**DISCUSSION**

These studies demonstrate that the CD ETB receptor is a physiological regulator of systemic BP. Deficiency of the ETB receptor in the CD causes hypertension on a normal-Na diet and elicits salt-sensitive hypertension associated with impaired ability to excrete a Na load. While these are the only studies selectively examining the function of CD ETB receptors in vivo, less specific analyses support these observations. Our findings are in agreement with studies utilizing in vivo administration of ETB receptor-specific agonists. When ET-1 is given in the setting of ETA, but not ETB, receptor blockade, a natriuretic effect is uncovered (5, 11). Specific ETB receptor agonists also increase urinary Na excretion (6, 52). An issue with these pharmacological studies is that ET agonists or antagonists invariably affect renal hemodynamics, making identification of a direct nephron effect problematic. ET-1, at 100 pM, inhibits sodium channel activity in A6 cells, while 10 nM ET-1 increases channel activity (14). Although not definitively studied, these concentrations suggest that the inhibitory effect of ET-1 is mediated by activation of ETB receptors (14). As discussed previously, whole animal knockout of the ETB receptor leads to amiloride-remediates salt-sensitive hypertension, although this cannot be primarily ascribed to the kidney (35). While no studies have examined the role of ETB receptors in mediating ET-1 inhibition of Na transport in isolated perfused CD, the ETB receptor has been demonstrated to mediate ET-1 inhibition of chloride flux in medullary thick ascending limb (40). Thus taken together, the bulk of indirect evidence supports the notion that renal ETB receptors exert a net natriuretic effect. The findings in the current study are the first to definitively implicate any nephron ETB receptor in causing natriuresis, as well as antihypertensive activity, in vivo.

The mechanisms by which CD ETB receptor activation reduces BP and promotes Na excretion are not fully known. In isolated perfused cortical CD, ET-1 inhibition of NaCl reabsorption is dependent on PKC activation and increases in intracellular Ca$^{2+}$ concentration (31, 32, 46). ET-1 reduction of Na reabsorption may involve Ca$^{2+}$ entry through dihydropyridine-type Ca$^{2+}$ channels associated with a sustained rise in intracellular Ca$^{2+}$ concentration (30, 34). Activation of the ETB receptor may inhibit Na-K-ATPase activity in the CD since ET-1, via the ETB receptor, increases CD PGE$_2$ production (29, 53) and the inhibitory effect of ET-1 on Na-K-ATPase activity in inner medullary CD suspensions is blocked by indomethacin (53). Nitric oxide (NO) may also be involved since ET-1, via the ETB receptor, increases CD NO production (42) and NO has been reported to inhibit CD amiloride-sensitive Na transport in CD (38). In the medullary thick ascending limb, the inhibitory effect of ET-1 on chloride flux is mediated by ETB receptor stimulation of NO production (40). Further exploration of the relevant mechanisms is needed.

The current study indicates that, while the CD ETB receptor is involved in regulation of BP and Na excretion, it does not fully account for the effects of CD-derived ET-1. BP is significantly greater in CD ET-1 KO mice on a normal-Na diet (as compared with their own controls), and these mice have substantially more salt-sensitive hypertension than CD ETA KO mice. In addition, CD ET-1 KO mice have greater Na retention, with grossly impaired Na excretion for up to 3 days after institution of high Na intake (1). The difference in BP and Na excretion between mice with CD-specific deletion of ET-1 and the ETB receptor is not due to the ETA receptor since CD ETA KO mice are not hypertensive and have no alterations in Na excretion on a normal- or high-Na diet (18). Thus CD-derived ET-1 must either be interacting with an as yet unknown ET receptor in the CD or is exerting effects on neighboring cells. An apparently unique ET-3 binding site that is inhibited by blockade with an ETA receptor antagonist has been reported.
in the medulla of spotted lethal rats with inherited deficiency of the ETB receptor, although the significance of this observation remains uncertain (44). CDs are adjacent to a number of structures which could be affected by CD-derived ET-1. In the outer medulla, ET-1 from CD could also inhibit thick ascending limb NaCl transport as discussed above. While ET-1 constricts isolated outer medullary descending vasa recta (39), intravenous ET-1 increases medullary blood flow via activation of vascular ETB receptors (2), an effect that would promote Na excretion (4, 13). Since this effect is mediated by NO and prostaglandins (9), it is conceivable that the greater salt sensitivity in CD ET-1 KO mice is due to a blunted NO or prostaglandin response. CD-derived ET-1 could also potentially bind to renal medullary interstitial cells; these cells, at least in culture, express both ETA and ETB receptors and also produce NO and prostaglandin E2 in response to ET-1 (55). Taken together, the above studies raise the strong possibility that CD-derived ET-1 exerts its antihypertensive and natriuretic effects through autocrine activation of CD ETB receptors as well as through paracrine mechanisms. The identity of such paracrine pathways requires further study, particularly since, at least to our knowledge, this is the first report implicating a tubule cell-derived factor acting on neighboring cells to exert physiological regulation of BP or Na excretion.

CD ETB KO mice had lower PRA on normal- and high-Na diets, and urinary aldosterone excretion on a normal-Na diet, than controls, consistent with expanded plasma volume in the knockout animals. In contrast, CD ET-1 KO mice, despite being more hypertensive and with greater Na retention, did not have reduced renin or aldosterone (1). The reasons why the renin-aldosterone axis is suppressed in CD ETB KO, but not CD ET-1 KO, mice are unknown. It is tempting to speculate that CD-derived ET-1, through paracrine mechanisms, may modulate release of substances from the kidney that downregulate the renin-aldosterone system. Further characterization of such a system would be of substantial importance.

In summary, the current study sheds light on a complex intrarenal system for regulation of urinary Na excretion and systemic BP. The CD increases ET-1 production, by as yet uncertain mechanisms, under conditions associated with extracellular fluid volume expansion (28). Such ET-1 causes autocrine inhibition, via the ETB receptor, of Na reabsorption. CD-derived ET-1 also likely exerts paracrine effects, conceivably through interaction with several cell types, which further promote a natriuresis and reduction of BP. These paracrine effects may also involve suppression of the renin-aldosterone axis. The CD ETA receptor does not appear to be involved in regulation of Na transport or BP. Taken together, these findings suggest that deficiency of the renal CD ET system could potentially have important implications in human hypertension. Initial studies indicate that urinary ET-1 excretion, which derives from renal ET-1 production, is reduced in patients with essential hypertension (20). Consequently, further exploration of this novel intrarenal system, as well as its potential derangement in hypertension, is highly warranted.

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