Role of endothelin-1 in renal regulation of acid-base equilibrium in acidoctic humans

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Pallini A, Hulter HN, Muser J, Krapf R. Role of endothelin-1 in renal regulation of acid-base equilibrium in acidoctic humans. Am J Physiol Renal Physiol 303: F991–F999, 2012. First published August 1, 2012; doi:10.1152/ajprenal.00309.2012.— Endothelin-1 inhibits collecting duct sodium reabsorption and stimulates proximal and distal tubule acidification in experimental animals both directly and indirectly via increased mineralocorticoid activity. Diet-induced acid loads have been shown to increase renal endothelin-1 activity, and it is hypothesized that increased dietary acid-induced endothelin-1 activity may be a causative progression factor in human renal insufficiency and that this might be reversed by provision of dietary alkali. We sought to clarify, in normal human volunteers, the role of endothelin-1 in renal acidification and to determine whether the effect is dependent on dietary sodium chloride. Acid-base equilibrium was studied in seven normal human volunteers with experimentally induced metabolic acidosis [NH4Cl 2.1 mmol·kg body weight (BW)−1·day−1] and with and without inhibition of endogenous endothelin-1 activity by the endothelin A/B-receptor antagonist bosentan (125 BID p.o/day) both during dietary NaCl restriction (20 mmol/day) and NaCl repletion (2 mmol NaCl·kg BW−1·day−1). During NaCl restriction, but not in the NaCl replete state, bosentan significantly increased renal net acid excretion in association with stimulation of ammoniagenesis resulting in a significantly increased plasma bicarbonate concentration (19.0 ± 0.8 to 20.1 ± 0.9 mmol/l) despite a decrease in mineralocorticoid activity and an increase in endogenous acid production. In pre-existing human metabolic acidosis, endothelin-1 activity worsens acidosis by decreasing the set-point for renal regulation of plasma bicarbonate concentration, but only when dietary NaCl provision is restricted.

ET-1 is an important physiological regulator of renal Na excretion and systemic blood pressure. ET-1, through activation of ET-B, has also been shown to stimulate proximal tubule acidification by enhancing proximal-tubular Na/H exchange (NHE3, via c-src activation). Distal tubule acidification in rats is also stimulated by systemic administration of ET-1, and the effect of ETs is mediated both by increased proton secretion and decreased HCO3− secretion (10, 11, 31, 32). Both indirect and direct effects might exist in ET-mediated distal acidification. In particular, ET has been reported to increase aldosterone activity (22) and to augment distal nephron acidification via this mechanism (12).

Evidence for homeostatic regulation of renal acidification by dietary acid-induced increases in renal endothelial ET-1 secretion has been reported in studies using microdialyzed renal cortical interstitial sampling in rats (32). Consistent with this hypothesis, increased proximal tubule proton secretory capacity has been reported in response to metabolic acidosis and was shown to be mediated, at least in part, by increased activation of ET-B (17, 18). Micropuncture studies in rat distal tubule have found that net acidification is also stimulated in these segments after a dietary acid load and that the increments are mediated in part by increased bosentan-inhibitable ET activity as modulated both by ET-induced apical Na/H exchange and by ET-induced hyperaldosteronism (12, 33).

While the authors did demonstrate higher plasma aldosterone levels in NaCl-restricted rats with higher acid loads from a high protein diet and that bosentan reduced the hyperaldosteronism, the micropuncture studies, including those showing an effect of spirinolactone, were performed with perfusate containing high concentrations of sodium (10). Since mineralocorticoid excess has no effect on renal or systemic acid-base equilibrium in NaCl-restricted acidoctic dogs with high acid loads (9), it is unclear how the micropuncture mineralocorticoid effects demonstrated with sodium present are related to the bosentan effects on net acid excretion (NAE) and acid production reported in salt-restricted rats (11, 12). These reported studies in rats are confounded further by the observation that such NaCl-restricted rats treated with prolonged bosantan administration exhibit decreased endogenous acid production (decreased NAE), such that it is unclear whether bosentan causes a primary decrease in distal H+ secretion or whether the bosentan-induced decreased distal tubule H+ secretion (irrespective of the issue of sodium in perfusate) simply reflects the requirement for decreased renal H+ secretion to adjust to a decreased H+ load requiring excretion, to maintain H+ balance (12, 33).

Whether and to what extent renal ET-1 mediates acidification in response to acid loads in humans, by proximal and/or distal mechanisms, has not been investigated. However, in
view of the ET-1 knockout data demonstrating an important effect on collecting duct ion transport (1) and the correlative evidence in rats among ET receptor agonism and the renal acid excretory response to dietary acid loads (10–12), investigation of the role of endogenous ET in humans is of great interest.

In view of the possibly major roles of ET-1 in tubular sodium and acid-base transport as well as its potential clinical role in mediating some of the clinical adverse effects of chronic acid loads, we wished to test in normal human subjects whether 1) ET-1 stimulates renal acidification in response to metabolic acidosis in humans, 2) ET-1 modulates the severity of metabolic acidosis resulting from a given acid load, 3) ET-1 regulates acidification in a sodium-dependent or sodium-independent manner, as judged by alterations in NaCl intake.

METHODS

Seven 24 (± 2) yr old normal male subjects (weight ± SD = 70 ± 3 kg) were studied for 4 wk under metabolic balance conditions. They ingested a constant diet during the entire study containing for every kilogram of body weight (BW) per day (in mmol): 1.41 potassium, 0.524 calcium, 0.73 phosphate (23 mg), and 16.9 nitrogen (0.24 g), as well as 44 ml water, and 37.2 kcal. As indicated in Fig. 1, the subjects ingested 20 mmol of sodium daily in the intrinsic diet during the two low-sodium diet periods and ingested a sodium-replete diet during the last two periods, when sodium chloride (2 mmol per kg BW daily) was added to the low-sodium diet. During all four study periods (see Fig. 1), we imposed an acid load consisting of oral ingestion of NH4Cl (in gelatin capsules) 2.1 mmol per kg BW per day, given in six evenly distributed doses over 24 h.

The effect of endogenous ETs on renal and systemic acid-base and electrolyte metabolism was studied with the oral nonselective ET-A/ET-B receptor antagonist bosentan (Actelion, Allschwil, Switzerland) at a dose of 125 mg BID (one dose every 12 h, see Fig. 1). This chronic dosing regimen is reported to provide a Cmax plasma level averaging 1,450 nM (30), a value well in excess of the reported IC50 values of 123 nM for ET-A and ~475 nM for ET-B in albumin solution (36, 37), although extrapolation of in vitro IC50 values to in vivo inhibitor levels can be imprecise. This regimen is reported to induce no significant changes in plasma renin activity, AII, and norepinephrine levels in subjects with essential hypertension (15).

Dietary 70 mmol of sodium daily in the intrinsic diet was divided doses over 24 h. Daily fasting arterialized venous samples were obtained from a heated forearm vein (5). Blood samples were accepted only if the partial pressure of oxygen exceeded 70 mmHg (9.3 kPa). Blood samples for plasma and whole blood (pH) analysis were obtained from heparinized syringes. Twenty-four hour urine samples were collected in plastic bottles containing mineral oil and thymol-chloroform preservative. During each study period a subject was considered to be in a steady-state when plasma values obtained on 3 consecutive replicates. Acid-base and electrolyte, ammonium, and phosphate values in blood and urine were determined as previously described (14).

Titratable acidity in urine was calculated from urinary phosphate excretion, urine pH, and blood pH with the pK’ of phosphate corrected for ionic strength by the method of Schwartz et al. (26). Plasma anion gap was calculated as Na+ – (Cl− + HCO3−). Urinary unmeasured anions were calculated as (Na+ + K+ + NH4+) – (Cl− + HCO3− + phosphate anion). Phosphate anion equivalency was calculated from corrected pK’ and urinary phosphate concentration. Urinary cortisol metabolites were determined by gas chromatography. Urinary tetrahydro-aldosterone was measured by radioimmunoassay.

Results are reported as means ± SE unless stated otherwise. Statistical significance was determined by ANOVA for repeated measurements.

The study protocol was approved by the ethics committee of both cantons of Basel (University of Basel, Basel, Switzerland). All subjects gave informed consent and were paid for their participation.

RESULTS

Figures 2 and 3 illustrate the daily blood and urine acid-base composition during all four study periods. The steady-state acid-base compositions of blood and urine at the end of these periods are depicted in Table 1, while corresponding steady-state plasma electrolytes, creatinine clearances, and body weights are shown in Table 2. During sodium restriction, ET-A/B inhibition increased renal acid excretion in association with increasing ammonium production (increase in urine pH and ammonium excretion), which resulted in a small, but significant increase in blood [HCO3−] from 19.0 ± 0.8 to 20.1 ± 0.9 mmol/l (P = 0.029). Thus, during dietary sodium restriction, ET-A/B inhibition attenuated metabolic acidosis by stimulation of renal acidification.

ET-A/B inhibition by oral bosentan under low-dietary NaCl salt resulted in a significant increase in endogenous acid production evidenced by the significant increase in steady-state renal NAE in period 2 compared with period 1 (168 vs. 150 meq/24 h, P < 0.05). Sodium repletion (study period 3, 2 mmol of NaCl per kg BW per day) resulted in a transient stimulation of renal acidification (NAE) and a further significant increase in blood [HCO3−] from 20.1 ± 0.9 to 21.2 ± 1.0 mmol/l. When ET-A/B inhibition (oral bosentan) was withdrawn (period 4), there was no significant effect on renal acid excretion nor on blood [HCO3−] (Table 1 and Figs. 2–4). In addition, steady-state NAE and thus endogenous acid production were similar during and after ET-A/B inhibition under salt-replete conditions (ET-A/B inhibition).
A/B inhibition in acidotic subjects under dietary sodium restriction.

As shown in Table 3, during period 3 (NaCl salt repletion and ET-A/B inhibition), there was an excess of sodium retained or an apparent relative urinary chloride loss. During this period, 242 mmol of sodium were retained, compared with only 117 mmol of chloride, in agreement with the increase in BW of +0.8 kg (Table 2). The difference (retained sodium minus retained chloride = 125 mmol, \( P = 0.01 \)) was accounted for, in large part, by both the increase in proton (i.e., NAE) excretion (+44 mmol for period 3, Fig. 4) and increased losses of K\(^+\) (+58 mmol for period 3).

Figure 5 shows that ET-A/B inhibition had no effect on urinary excretion of cortisol metabolites but significantly inhibited the urinary excretion and, thus the adrenal production rate, of aldosterone during sodium restriction as measured by the excretion of the tetrahydro-metabolite. No effect of ET-A/B inhibition on aldosterone excretion was demonstrable during sodium repletion. This provides confirmatory evidence for the previous observation that ET stimulates aldosterone production (19) and strong new evidence for the notion that the stimulatory effect of ET-A/B inhibition on renal acidification during sodium restriction is independent of aldosterone activity.

**Other observations: effects of ET-A/B inhibition on blood pressure, uric acid, and calcium metabolisms.** Under low-NaCl salt intake and acidosis, bosentan significantly decreased both systolic and diastolic blood pressures (Table 4). NaCl repletion in period 3 resulted surprisingly in a further small, but significant decrease in blood pressure (Table 4), which increased significantly when bosentan was discontinued in period 4.

**Period 4 values** (acidosis and salt repletion) were not different from baseline values in period 1 (acidosis and low-salt diet).

Plasma uric acid concentration decreased significantly and reversibly during ET-A/B inhibition in both the sodium-restricted and sodium-repleted state (Table 2). While plasma total and ionized calcium concentrations were not affected by ET-A/B inhibition, bosentan increased renal calcium excretion by inhibiting renal tubular calcium reabsorption (increase in the fractional excretion rate of calcium, Table 5). The effect was independent of dietary sodium and occurred with no measurable changes in intact parathyroid hormone (PTH) and 1,25(OH)\(_2\)D\(_2\) serum concentrations (Table 6).

**DISCUSSION**

These studies in humans evaluated the effects of ET-A/B inhibition by bosentan on renal acidification in pre-existing chronic metabolic acidosis (induced by ingestion of NH\(_4\)Cl) under the conditions of NaCl salt restriction and NaCl salt repletion. The key findings were:

1. **ET-A/B inhibition by bosentan, under low-salt conditions,** stimulated renal acidification with sufficient potency to increase plasma bicarbonate concentration despite an increase in the endogenous production rate of acid requiring renal excretion and thereby attenuated the severity of acidosis. The increased NAE was associated with increased renal ammonia production (urine pH increased), such that the increase in renal NH\(_3\) excretion was not attributable to increased trapping of an unchanged NH\(_3\) supply.

2. In response to NaCl repletion, renal NAE was further stimulated independently of ET-A/B inhibition (lack of revers-
The ability after discontinuation of bosentan), and the renal NaCl response was associated with significantly lower chloride than sodium retention, i.e., relative renal chloride loss.

3) The ability of ET-A/B inhibition to stimulate NAE with an associated increase in plasma bicarbonate concentration distinguishes the ET-1 inhibitory effect from that of mineralocorticoid excess inasmuch as a low-NaCl diet completely prevents any NAE and plasma bicarbonate effects during chronic acidosis (9).

4) ET-A/B inhibition decreases both systolic and diastolic blood pressures under salt-restricted and salt-replete conditions in normotensive subjects.

The finding that chronic bosentan administration to acidotic subjects on a low-NaCl diet was able to partially correct acidosis by a renal mechanism suggests the operation of a mechanism that is independent of distal nephron sodium transport-driven renal acidification. The magnitude of this effect is also sufficient to override a simultaneous increase in extrarenal endogenous acid production. This observation contrasts with the results in rats where bosentan elicited a decrease in extrarenal acid production, a finding which, in itself, might explain the bosentan-induced decrease in distal acidification in that species (10, 23). The reason(s) for this discrepancy between species are not apparent. The possibility that a correction of acidosis occurs with prolonged acid loading when a low-NaCl diet is ingested (i.e., spontaneously and independently of bosentan) is minimized by the finding of a rigorously demonstrated unchanged steady state in plasma \( \text{HCO}_3^-/\text{H}_2\text{CO}_3 \) under such conditions in HCl-treated dogs over an interval of 21 days (4).

NaCl repletion in the acidotic subjects with continued administration of bosentan in period 3 induced a transient increase in NAE (largely increased ammonium excretion), resulting in a further significant increase in the plasma \( \text{HCO}_3^- \) from 20.1 to 21.2 mmol/l. NAE then returned to values approximating those of the steady-state values of period 1 (Figs. 2 and 4), suggesting that the effect on extrarenal acid production.

Fig. 3. ET-A/B inhibition by oral bosentan during both low-NaCl and NaCl-replete conditions in NH4Cl-induced metabolic acidosis. Effect on 24 h urinary net acid excretion (NAE) rates, 24 h urinary ammonium (NH4) excretion, and urinary pH (pH U). *P < 0.05 (see text for details).

Table 1. Effect of endothelin inhibition by bosentan during low-salt diet and salt-repletion acidosis on steady-state blood and urinary acid-base composition

<table>
<thead>
<tr>
<th>Condition</th>
<th>Blood pH, U</th>
<th>HCO3(^{-}), mmol/l</th>
<th>PaCO2, mmHg</th>
<th>Urinary pH, U</th>
<th>NH4(^{+}), mmol/24 h</th>
<th>Titratable Acidity, mmol/24 h</th>
<th>Net Acid, mmol/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>7.347 ± 0.016</td>
<td>19.0 ± 0.8</td>
<td>35.8 ± 1.8</td>
<td>5.15 ± 0.11</td>
<td>125 ± 15</td>
<td>25 ± 3</td>
<td>150 ± 14</td>
</tr>
<tr>
<td>Low-salt, acidosis, +bosentan</td>
<td>7.375 ± 0.014(^{*})</td>
<td>20.1 ± 0.9(^{*})</td>
<td>35.6 ± 1.4</td>
<td>5.35 ± 0.15(^{*})</td>
<td>150 ± 17(^{*})</td>
<td>18 ± 3</td>
<td>168 ± 17(^{*})</td>
</tr>
<tr>
<td>Replete-salt acidosis, +bosentan</td>
<td>7.381 ± 0.015(^{*})</td>
<td>21.2 ± 1.0(^{*})</td>
<td>37.0 ± 1.5</td>
<td>5.11 ± 0.11(^{*})</td>
<td>135 ± 18</td>
<td>21 ± 3</td>
<td>156 ± 19</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>7.387 ± 0.010</td>
<td>21.6 ± 0.9</td>
<td>37.2 ± 1.6</td>
<td>5.05 ± 0.15</td>
<td>137 ± 11</td>
<td>20 ± 3</td>
<td>157 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SE. For definition of study periods see METHODS and Fig. 1. *P < 0.05 for comparison with previous steady-state period. Urinary HCO3\(^{-}\) 24 h excretion rates were <0.5 mmol in every subject and are not shown here nor were they included in the calculation of net acid excretion.
Table 2. Effect of endothelin inhibition by bosentan during low-salt diet and salt-repletion acidosis on steady-state blood acid base parameters, plasma electrolytes, creatinine, and body weights.

<table>
<thead>
<tr>
<th>Unmeasured Anions</th>
<th>Na⁺, mmol/l</th>
<th>K⁺, mmol/l</th>
<th>PO₄, mmol/l</th>
<th>Mg²⁺, mmol/l</th>
<th>Uric Acid, mmol/l</th>
<th>Creatinine, mg/dl</th>
<th>Body weight, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No acidosis</td>
<td>156 ± 2</td>
<td>3.9 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>118 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>60.7 ± 2.1</td>
</tr>
<tr>
<td>Acidosis</td>
<td>157 ± 2</td>
<td>4.0 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>120 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>60.4 ± 2.0</td>
</tr>
<tr>
<td>Acidosis + bosentan</td>
<td>138 ± 3</td>
<td>3.8 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>117 ± 0.6</td>
<td>1.5 ± 0.1</td>
<td>59.0 ± 1.8</td>
</tr>
</tbody>
</table>

Table 3. Relative sodium retention (or relative chloride loss) in period 3.

<table>
<thead>
<tr>
<th>Ion Retention</th>
<th>Na⁺, mmol</th>
<th>Cl⁻, mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>131</td>
<td>112</td>
</tr>
<tr>
<td>Day 2</td>
<td>79</td>
<td>40</td>
</tr>
<tr>
<td>Day 3</td>
<td>79</td>
<td>-11</td>
</tr>
<tr>
<td>Day 4</td>
<td>-7</td>
<td>-4</td>
</tr>
<tr>
<td>Day 5</td>
<td>-7</td>
<td>-13</td>
</tr>
<tr>
<td>Day 6</td>
<td>-15</td>
<td>-5</td>
</tr>
<tr>
<td>Day 7</td>
<td>-3</td>
<td>-2</td>
</tr>
<tr>
<td>Cumulative sum</td>
<td>242</td>
<td>117</td>
</tr>
<tr>
<td>Difference</td>
<td>Na⁺ - Cl⁻</td>
<td></td>
</tr>
</tbody>
</table>

Within period 3, the daily change from period 2 for sodium and chloride retention was calculated by subtracting the observed period 3 steady-state excretion values from the observed daily ion excretion values within period 3 for each subject, to adjust for the change from a low-NaCl diet to NaCl repletion. *Statistically significant from zero with P = 0.01.

Fig. 4. ET-A/B inhibition by oral bosentan during both low-NaCl and NaCl-replete conditions in NH4Cl-induced metabolic acidosis. Within period sums of daily changes in NAE relative to the mean value of the previous steady-state period are depicted for periods 2–4. Mean NAE for each period is shown as vertical bars.
ENDOTHELIN-1 AND RENAL ACID EXCRETION

Table 4. Effect of bosentan on mean 24 h systolic and diastolic blood pressures and heart rate in acidicotic, normotensive human subjects on a low-NaCl and a NaCl-replete diet

<table>
<thead>
<tr>
<th></th>
<th>Mean Systolic Blood Pressure, mmHg</th>
<th>Mean Diastolic Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>112 ± 5</td>
<td>72 ± 4</td>
<td>68 ± 5</td>
</tr>
<tr>
<td>Low-salt acidosis, +bosentan</td>
<td>107 ± 4*</td>
<td>65 ± 5*</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Replete-salt acidosis, +bosentan</td>
<td>105 ± 4†</td>
<td>64 ± 5†</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>108 ± 6*</td>
<td>66 ± 4</td>
<td>67 ± 4</td>
</tr>
</tbody>
</table>

*p < 0.05 for the comparison with previous steady-state period. †P < 0.05 for the comparison with low salt with acidosis period. For definition of study periods see see METHODS and Fig. 1.

Table 4. Effect of bosentan on mean 24 h systolic and diastolic blood pressures and heart rate in acidicotic, normotensivehuman subjects on a low-NaCl and a NaCl-replete diet.
Table 5. Effect of endothelin inhibition by bosentan during low-salt diet and salt-repletion acidosis on steady-state 24 h urinary electrolyte, uric acid, urea, and ionic fractional excretion rates

<table>
<thead>
<tr>
<th></th>
<th>Na⁺, mmol/24 h</th>
<th>K⁺, mmol/24 h</th>
<th>Cl⁻, mmol/24 h</th>
<th>Ca²⁺, mmol/24 h</th>
<th>PO₄, mmol/24 h</th>
<th>Mg²⁺, mmol/24 h</th>
<th>Unmeasured Anions, mmol/24 h</th>
<th>Uric Acid, mmol/24 h</th>
<th>Urea, mmol/24 h</th>
<th>FENa, %</th>
<th>FEK, %</th>
<th>FECa, %</th>
<th>FEPO₄, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>18 ± 1</td>
<td>106 ± 8</td>
<td>160 ± 10</td>
<td>8.0 ± 1.1</td>
<td>31.5 ± 3.5</td>
<td>6.8 ± 0.8</td>
<td>48 ± 9</td>
<td>3.44 ± 0.27</td>
<td>482 ± 22</td>
<td>0.17 ± 0.04</td>
<td>26.9 ± 2.1</td>
<td>2.42 ± 0.32</td>
<td>25.4 ± 2.4</td>
</tr>
<tr>
<td>+ bosentan</td>
<td>17 ± 1</td>
<td>88 ± 9</td>
<td>170 ± 11</td>
<td>9.6 ± 1.4</td>
<td>25.0 ± 2.5</td>
<td>6.2 ± 0.9</td>
<td>53 ± 9</td>
<td>3.37 ± 0.22</td>
<td>462 ± 21</td>
<td>0.09 ± 0.06</td>
<td>19.3 ± 1.3</td>
<td>2.78 ± 0.28</td>
<td>17.8 ± 1.8</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>160 ± 6</td>
<td>92 ± 7</td>
<td>319 ± 23</td>
<td>9.8 ± 1.3</td>
<td>26.5 ± 2.4</td>
<td>6.3 ± 0.7</td>
<td>35 ± 8</td>
<td>3.45 ± 0.25</td>
<td>386 ± 26</td>
<td>0.93 ± 0.13</td>
<td>19.9 ± 2.4</td>
<td>2.90 ± 0.25</td>
<td>17.1 ± 2.1</td>
</tr>
<tr>
<td>+ bosentan</td>
<td>172 ± 7</td>
<td>96 ± 7</td>
<td>325 ± 24</td>
<td>8.6 ± 1.0</td>
<td>27.0 ± 2.0</td>
<td>6.6 ± 0.9</td>
<td>45 ± 9</td>
<td>3.60 ± 0.28</td>
<td>383 ± 22</td>
<td>1.01 ± 0.14</td>
<td>20.9 ± 2.1</td>
<td>2.60 ± 0.28</td>
<td>17.4 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. For definition of study periods see METHODS and Fig. 1. *P < 0.05 for comparison with previous steady-state period.

Table 6. Effect of bosentan on steady-state serum concentration of intact PTH and 1,25(OH)₂D

<table>
<thead>
<tr>
<th></th>
<th>Intact PTH, pmol/l</th>
<th>1,25(OH)₂D, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>2.7 ± 0.3</td>
<td>5.9 ± 3.2</td>
</tr>
<tr>
<td>+ bosentan</td>
<td>6.9 ± 0.1</td>
<td>8.4 ± 1.4</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>2.8 ± 0.5</td>
<td>5.1 ± 4.9</td>
</tr>
<tr>
<td>+ bosentan</td>
<td>5.0 ± 0.5</td>
<td>5.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. PTH, parathyroid hormone.
20, 24) have shown that amelioration of metabolic acidosis by oral citrate or bicarbonate or encouragement of an alkaline-producing diet (increasing fruit and vegetable intake) simultaneously decreased renal ET-1 excretion and nominally slowed the decrease in estimated GFR, suggesting that acidosis-induced stimulation of renal ET-1 production may be an important factor in the progressive fall in GFR in patients with CKD. However, all of the human CKD data were limited to serum cystatin C or creatinine-based GFR estimations, and none of these studies has demonstrated a significant attenuation of the intergroup (placebo vs. alkali) rate of estimated GFR decline.

Our finding that normal subjects on an ET-A/B inhibitor were capable of responding to the imposed 2 mmol/kg increase in NaCl intake without abnormal or progressive Na retention is of interest inasmuch as placebo-controlled clinical trials of such agents (ET-A/B and selective ET-1 A inhibitors) in hypertension and CKD have reported delayed trends (after weeks of treatment) of increased congestive heart failure (CHF) events, “fluid overload” events, weight gain, and decreases in blood hemoglobin concentration (19, 21). This difference may have clinical importance since patients with hypertension and CKD are more susceptible to CHF events; ET-1 inhibitors conceivably might be associated with such clinical events via primary cardiovascular effects rather than by primary renal transport effects.

In summary, based on the effects of ET-A/B inhibition in this study in normal human subjects with pre-existing NH4Cl-induced metabolic acidosis, it is concluded that: 1) ET-1 decreases the set point for renal regulation of plasma bicarbonate concentration during acidosis in humans inasmuch as bosentan increased plasma bicarbonate concentration during NaCl-restricted conditions despite its effect of increasing endogenous acid production; 2) No effect of ET-1 was demonstrated on renal or systemic acid-base metabolism under NaCl-replete conditions; 3) ET-1 apparently exerts important modulating effects on renal chloride reabsorption affecting renal and systemic acid-base equilibrium and, possibly, blood pressure. The mechanism(s) of this effect requires further studies; 4) ET-1 inhibition with bosentan decreases plasma uric acid concentration in acidiotic humans at least in part as a consequence of decreased renal uric acid clearance; 5) ET-1 inhibits renal ammoniagenesis and, thereby, might accentuate metabolic acidosis during NaCl-restricted conditions, if other urinary buffers were deficient.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


