Role of endothelin-1 in renal regulation of acid-base equilibrium in acidic 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 acidic 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] 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 mol 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.


While known chiefly as vasoconstrictors, ETs are also reported to modulate renal sodium reabsorption and renal proton secretion (25). ET-1, through activation of ET-B, inhibits collecting duct sodium and water reabsorption. The finding that collecting duct-specific knockout of ET-1 causes hypertension and sodium retention (1) provides strong evidence that renal 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 have 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 spirronolactone, 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 acidic 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 bosentan 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

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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 meta-
abolic acidosis resulting from a given acid load, 3) ET-1
regulates acidification in a sodium-dependent or sodium-inde-
pendent 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
dynamic 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

\[ \text{IC}_{50} = 123 \text{nM for ET-A} \]

\[ \text{IC}_{50} = 123 \text{nM for ET-B} \]

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).

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-chlo-
roform preservative. During each study period a subject was considered
to be in a steady-state when plasma values obtained on 3 consecutive
days varied by no more than 1.5 mmol/l for bicarbonate.

Ambulatory systolic and diastolic blood pressure and heart rate
were recorded on 4 days (at the end of each of the four study periods)
during 24 h with measurements at 15 min intervals. (Spacelabs
Ambulatory BP Monitor, model 90217; Spacelabs Healthcare, Issa-
qua, WA). Values reported are 24 h mean values.

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 in-
creased renal acid excretion in association with increasing
ammonia production (increase in urine pH and ammonium excre-
tion), 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 inhibi-
tion attenuated metabolic acidosis by stimulation of renal acidif-
ication.

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
NAE in period 2 compared with period 1 (168 vs. 150
meg/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-

![Fig. 1. Diagram of study protocol.](http://ajprenal.physiology.org/doi/pdf/10.1152/ajprenal.00309.2012)
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)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\(_4\) 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-
ibility 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 [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 produc-

![Figure 3. ET-A/B inhibition by oral bosentan during both low-NaCl and NaCl-replete conditions in NH\(_4\)Cl-induced metabolic acidosis. Effect on 24 h urinary net acid excretion (NAE) rates, 24 h urinary ammonium (NH\(_4\)) excretion, and urinary pH (pH U). *P < 0.05 (see text for details).](attachment:image.png)

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>Blood pH, U</th>
<th>HCO(_3^-), mmol/l</th>
<th>P(_{\text{ACO}}), mmHg</th>
<th>Urinary pH, U</th>
<th>NH(_4^+), 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>
</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>
</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>
</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>
</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 HCO\(_3^-\) 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>Ion Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;, mmol</td>
</tr>
<tr>
<td><strong>Day 1</strong></td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
</tr>
<tr>
<td><strong>Day 6</strong></td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
</tr>
<tr>
<td><strong>Cumulative sum</strong></td>
</tr>
<tr>
<td><strong>Difference (Na&lt;sup&gt;+&lt;/sup&gt; - Cl&lt;sup&gt;-&lt;/sup&gt;)</strong></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 F<sub>0.05</sub>.

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

| Ion Retention | Na<sup>+</sup>, mmol | Cl<sup>-</sup>, mmol |
|---------------|
| **Day 1** | 131 | 112 |
| **Day 2** | 64 | 40 |
| **Day 3** | 79 | 11 |
| **Day 4** | 7 | -4 |
| **Day 5** | 7 | -13 |
| **Day 6** | -15 | -5 |
| **Day 7** | -3 | -2 |
| **Cumulative sum** | 242 | 117 |
| **Difference (Na<sup>+</sup> - Cl<sup>-</sup>)** | 125<sup>*</sup> |

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 F<sub>0.05</sub>.

Fig. 4. ET-A/B inhibition by oral bosentan during both low-NaCl and NaCl-replete conditions in NHCl-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.

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Chronic metabolic acidosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Low Sodium</th>
<th>Sodium Repletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>bosentan</td>
<td>bosentan</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>bosentan</td>
<td>bosentan</td>
</tr>
</tbody>
</table>

than sodium or relative chloride underretention, Table 3). The relative (relative to the amount of sodium retained) chloride (anion) underretention of 125 mmol was accounted for in large part by the cumulative increase in NAE and the cumulative loss of potassium (44 + 58, respectively = 102 mmol) in period 3. This relative chloride-to-sodium loss could have been responsible for a part of the rise in plasma \([\text{HCO}_3^-]\) via a relative contraction of the chloride (extracellular fluid) space.

The present results with bosentan do not exclude a primary increase in proximal tubule acidification as the primary effect causing delivery of a relatively bicarbonate-poor luminal fluid to distal sites, enabling a transient increase in distal buffered acid excretion (TA and \(\text{NH}_4\)), even at a constant distal \(\text{H}^+\) secretion rate. Alternatively, the mechanism could be a primary mineralocorticoid-independent (9) effect on collecting duct \(\text{H}^+\) secretion or bicarbonate reabsorption. Thus an effect of bosentan to stimulate collecting duct \(\text{H}^+\)-ATPase by an occult mechanism remains possible, but such a pure proton secretory effect in the absence of a simultaneous increase in lumen-negative voltage, as with mineralocorticoid, would be unlikely in view of the mineralocorticoid data in dogs.

The present results are inconsistent with ET-1’s augmenting proximal and distal acidification in rodents (10,11, 31, 32). The opposite effects of bosentan on rodent vs. human endogenous acid production also remain occult, but the finding of opposite interspecies effects acting in tandem for both parameters suggests that the ET system may be a relatively important factor in control of acid-base equilibrium in both species.

The fact that discontinuation of bosentan in period 4 did not result in acid retention (decrease in NAE) and a decrease in plasma bicarbonate concentration with a return to values approaching those in period 1 raises the possibility that humans with chronic mineral acidosis might exhibit larger diet NaCl-dependent differences in plasma bicarbonate concentration than those reported in dogs (4).

While it is known that in nonacidotic humans plasma \([\text{HCO}_3^-]\) varies inversely with the amount of dietary NaCl (2), at least when acid loads are low, there are, surprisingly, no reported human studies that have examined the dependence of severity of metabolic acidosis on dietary NaCl intake for acid loads comparable to those of the mineral acidosis employed in this study. Nevertheless, de Sousa et al. (4) observed a tendency for smaller decreases in plasma \([\text{HCO}_3^-]\) in response to mineral acid loads (HCl) in dogs under a normal NaCl intake than in dogs on a zero NaCl diet. However, provision of chloride alone with no other maneuver caused acidosis in Na-void dogs with a large decrease in NAE (28). Whether those diet chloride-dependent changes in NAE and plasma bicarbonate concentration were due to altered distal sodium delivery or to effects of changes in chloride concentration in the lumen of the distal nephron or other factors is not known. The answer to the question as to whether an appreciable effect of diet NaCl on plasma \([\text{HCO}_3^-]\) might occur in chronically acidic human subjects without ET-A/B inhibition is not clear and should be addressed in an appropriately designed study.

**Blood pressure effects.** ET-AB inhibition by bosentan decreased systolic and diastolic blood pressures similarly in both NaCl salt restriction and repletion (Table 4) and is evidence for a systemic vasodilatory effect of bosentan as more important than the ET-A/B inhibitory effect on both proximal and distal

Table 4. Effect of bosentan on mean 24 h systolic and diastolic blood pressures and heart rate in acidic, 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 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>Ca2+, mmol/24 h</th>
<th>Mg2+, mmol/24 h</th>
<th>Anions, mmol/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>18.0 ± 2.1</td>
<td>2.4 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>18.0 ± 2.1</td>
<td>2.4 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td>0.3 ± 0.3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Intact PTH, pmol/l</th>
<th>1,25(OH)2 Vitamin D, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt acidosis</td>
<td>2.7 ± 0.3</td>
<td>66.0 ± 6.1</td>
</tr>
<tr>
<td>Low-salt acidosis, +bosentan</td>
<td>2.2 ± 0.5</td>
<td>56.0 ± 5.9</td>
</tr>
<tr>
<td>Replete-salt acidosis, +bosentan</td>
<td>2.8 ± 0.5</td>
<td>52.1 ± 6.4</td>
</tr>
<tr>
<td>Replete-salt acidosis</td>
<td>3.2 ± 0.6</td>
<td>49.8 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. PTH, parathyroid hormone.

tubule sodium and chloride reabsorption, at least during NaCl restriction. In collecting duct conditional ET-1 knockout mice (1), however, there was an exaggerated rise in blood pressure under high-NaCl salt provision, which is contrary to our finding of a decrease in blood pressure in response to a NaCl intake increase during bosentan administration. The overall effect of bosentan on blood pressure in the present observations suggests a predominance of systemic vasodilatory effects over the renal salt-reabsorptive effects in these acidotic human subjects. The small, but significant decline in blood pressure during ET-A/B inhibition despite and in response to increased dietary NaCl salt (period 3) is surprising and not readily explained. It is possible that the further decrease in aldosterone activity in period 3 (Fig. 5) and the relative loss of chloride to sodium in period 3 (Table 3) were operative in this response. There is evidence that the blood pressure response to sodium is critically dependent on chloride as the accompanying anion provided (16).

Effects of ET-A/B inhibition on renal uric acid and calcium handling in acidic subjects. Uric acid plasma concentrations decreased significantly and reversibly during ET-A/B inhibition in both the sodium-restricted and sodium-repleted state (Table 2), consistent with recent data in human patients with pulmonary hypertension, although those patients also demonstrated improvements in heart failure (29). Since renal uric acid clearance increased in response to ET-A/B inhibition (Tables 2 and 5), bosentan either increases tubular uric acid secretion or inhibits uric acid reabsorption. The mechanisms underlying these observations were not clarifiable during the present studies. Calcium and magnesium homeostasis [and PTH and 1,25(OH)2D levels, Table 6] were not appreciably affected by ET-A/B inhibition, but fractional excretion of calcium was enhanced both in low- and high-NaCl conditions. The cellular mechanism of this effect is unknown. The mechanism for the small but significant bosentan-induced decrease in urinary phosphate excretion under salt-restricted conditions is also not apparent. The mechanism for the fall in urinary urea excretion under salt-replete conditions, irrespective of bosentan administration, is also not apparent in the absence of stool nitrogen data.

Dietary acid loads have been suggested to correlate with the progressive decline in glomerular filtration rate (GFR) in rats with reduced renal mass and humans with chronic kidney disease (CKD) (23, 24, 35). The GFR-acid correlative effect has been suggested to be mediated at least in part by the effects of local acidity to increase renal ET-1 production with secondary induction of tubule-interstitial injury (20). Interpretation has been further complicated by difficulties in controlling for blood pressure changes. Recent studies in CKD patients (3, 8,
20, 24) have shown that amelioration of metabolic acidosis by oral citrate or bicarbonate or encouragement of an alkali-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 acidic 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


