The Renin-Angiotensin and Renal Dopaminergic Systems Interact in Normotensive Humans

Aruna R. Natarajan,* Gilbert M. Eisner,† Ines Armando,‡ Shaunagh Browning,§ John C. Pezzullo,§ Lauren Rhee,§ Mustafa Dajani,* Robert M. Carey,| and Pedro A. Jose‡¶

Departments of *Pediatrics and †Internal Medicine, MedStar-Georgetown University Hospital, Washington, DC; ‡Department of Medicine, Division of Nephrology, and ¶Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland; §Clinical Research Unit, Georgetown University Medical Center, Washington, DC; and |Department of Internal Medicine, The University of Virginia, Charlottesville, Virginia

ABSTRACT

The renin-angiotensin-aldosterone (RAAS) and renal dopaminergic systems interact to maintain sodium balance. High NaCl intake increases renal synthesis of dopamine and dopaminergic receptor activity, decreasing epithelial sodium transport, whereas sodium deficit activates the RAAS, increasing epithelial sodium transport. We tested the hypothesis that attenuation of the natriuretic effect of dopamine D1-like receptors during salt restriction results in part from increased RAAS activity in seven salt-resistant normotensive adults using a double-blind placebo-controlled balanced crossover design. All subjects attained sodium balance on low (50 mmol Na+/day) and high (300 mmol Na+/day) NaCl diets, administered 4 weeks apart. Sodium, potassium, lithium, para-aminohippurate, and creatinine clearances were measured before, during, and after a 3-hour infusion of fenoldopam, a D1-like receptor agonist, with and without pretreatment with enalapril, an angiotensin converting enzyme inhibitor. On the high NaCl diet, fenoldopam-induced natriuresis was associated with the inhibition of renal proximal and distal tubule sodium transport. On the low NaCl diet, fenoldopam decreased renal distal tubule sodium transport but did not cause natriuresis. The addition of enalapril to fenoldopam restored the natriuretic effect of fenoldopam and its inhibitory effect on proximal tubule sodium transport. Thus, on a high NaCl diet fenoldopam causes natriuresis by inhibiting renal proximal and distal tubule sodium transport, but on a low NaCl diet the increased RAAS activity prevents the D1-like receptor from inhibiting renal proximal tubule sodium transport, neutralizing the natriuretic effect of fenoldopam. These results demonstrate an interaction between the renin-angiotensin and renal dopaminergic systems in humans and highlight the influence of dietary NaCl on these interactions.


During salt depletion, sodium balance is maintained by increased activity of several systems, including the renin-angiotensin-aldosterone (RAAS) and sympathetic nervous systems.1–3 During salt loading, the RAAS and sympathetic nervous system are inhibited,4 while pressure-natriuresis5 and natriuretic hormones/factors such as adrenomedullin,6 angiotensin-(1–7),7 angiotensin III,8 atrial natriuretic peptide,9 eicosanoids,10 endothelin,11,12 nitric oxide,13 ouabain,14 prolactin,15 urodilatin,16 and intrarenal dopamine,17 among others, are operative. Direct/indirect activation of the intrarenal dopaminergic system causes at least 50% of sodium excretion during salt loading17–20 by decreasing sodium transport in the proximal tubule,21,22 thick ascending limb,23 and more distal segments of the
nephron.\textsuperscript{22–24} During salt restriction, the ability of renal endogenous dopamine to inhibit sodium transport is abolished.\textsuperscript{22} This phenomenon is attributable to decreased renal dopamine production, altered dopamine receptor subtype expression, and postreceptor mechanisms in the renal tubule, and to overriding effects of salt-conserving mechanisms such as the sympathetic nervous system, the RAAS, and other salt-retaining hormones.

The RAAS, via angiotensin type 1 (AT\textsubscript{1}) receptors, and the intrarenal dopaminergic system, via D\textsubscript{1}-like and D\textsubscript{2}-like receptors, exert counter regulatory effects on sodium balance.\textsuperscript{24–28} Dopamine can also negatively regulate renin production,\textsuperscript{29} while angiotensin II (AT\textsubscript{2}) can increase dopamine turnover.\textsuperscript{30} By contrast, D\textsubscript{1}-like and AT\textsubscript{2} receptors cooperate to inhibit renal proximal sodium transport,\textsuperscript{31} causing natriuresis.\textsuperscript{32} In the current study, we tested the hypothesis that the previously reported attenuated natriuretic effect of D\textsubscript{1}-like receptor agonists in normotensive humans\textsuperscript{22} during salt restriction is caused, in part, by increased RAAS activity. In this double-blind, placebo-controlled, balanced crossover study, we examined the effect of inhibition of the angiotensin converting enzyme with enalapril on the natriuretic effect of D\textsubscript{1}-like receptor stimulation with fenoldopam in salt-resistant normotensive subjects on low-salt (LS) and high-salt (HS) diets. We now show, for the first time in humans, that on a HS diet, fenoldopam causes natriuresis by inhibition of proximal and distal sodium transport, but on a LS diet, increased activity of the renin-angiotensin system (RAS) contributes to the inability of D\textsubscript{1}-like receptors to inhibit proximal tubule sodium transport and impairs the inhibitory effect of fenoldopam on distal sodium transport, preventing natriuresis.

RESULTS

Recruitment and Baseline Characteristics

Of 93 volunteers, 19 eligible subjects were screened and enrolled after informed consent (Supplemental Materials 1–3). Seventeen subjects started the diet. Four were excluded for noncompliance and one for sinus tachycardia and facial flushing during the fenoldopam infusion. Eight subjects completed both phases of the trial. Seven normotensive salt-resistant subjects were included, while one normotensive salt-sensitive subject (\(>10\%\) increase in mean BP after 5 days on the HS diet)\textsuperscript{33} was excluded from data analysis (Figure 1, Table 1).

All subjects attained sodium balance with urinary sodium excretion of 52.8\(\pm\)7.4 mmol/24 h on Day 5 of the LS diet (50 mmol/24 h) and 296.5\(\pm\)10.9 mmol/24 h on Day 5 of the HS diet (300 mmol/24 h). Urinary dopamine on Day 5 was higher on the HS than the LS diet (Figure 2A). Fenoldopam was started when subjects were in a steady state of diuresis attained 3.5–4 hours after an oral water load of 20 ml/kg.

The parameters described below were measured at half-hourly intervals (Figure 2B).

Cardiovascular and Renal Hemodynamic Parameters in Response to Fenoldopam, Enalapril, or the Combination of Fenoldopam and Enalapril

Heart rates and systolic BP\textsuperscript{22,34} on LS and HS diets were similar and unchanged with fenoldopam,\textsuperscript{22,34} enalapril, or the combination of fenoldopam and enalapril. Diastolic and mean BPs were similar at baseline on both salt diets establishing the salt-resistant phenotype.\textsuperscript{33,35–37} Fenoldopam\textsuperscript{34} alone decreased diastolic and mean BPs on HS diets and decreased diastolic BP on LS diets, in contrast to its ability to lower systolic and diastolic BPs in mildly and moderately hypertensive patients.\textsuperscript{38} Fenoldopam and enalapril given together decreased diastolic and mean BPs on both salt diets (Table 2).

![Figure 1. Cohort recruitment and retention for study. Of the 93 responders to public advertisements, 45 subjects underwent physical examination; 17 subjects went on trial. Eight subjects completed both HS and LS diet phases 4 weeks apart. One salt-sensitive subject was excluded from the final evaluation of the data. *Obesity: BMI >30 (males), BMI >28.6 (females). **Prehypertension: BP 120–139/80–89 JNC VII (2003). BMI, body mass index.](jasn.27.265-279.f1)
Basal effective renal plasma flow (RPF), estimated by paraaminohippurate (PAH) clearance (ml/min per 1.73 m²), was similar on the LS and HS diets, and increased in response to fenoldopam alone on HS but not LS diets (Table 3). Enalapril did not affect RPF but prevented the increase in PAH clearance caused by fenoldopam on the HS diet.

The baseline filtration fraction (FF) was similar and unchanged by fenoldopam and enalapril alone or in combination on both salt diets. Transient decreases in FF were observed at the start of the fenoldopam infusion on the HS diet and the combination of enalapril and fenoldopam on the LS diet (data not shown).

Renal Clearances

Creatinine Clearance and Filtered Sodium Load
Basal creatinine clearance (estimate of GFR) and filtered sodium load were unaffected by salt intake. On LS and HS diets, creatinine clearance and filtered sodium load did not change with fenoldopam, enalapril, or the combination of fenoldopam and enalapril. However, both creatinine clearance and filtered sodium load with fenoldopam and enalapril were higher on the HS than the LS diet (Table 4A).

Urine Flow
Urine flow (ml/min) was similar on both salt diets. Diuresis occurred with fenoldopam alone38 and fenoldopam combined with enalapril on the LS and HS diets. The percent increase in the urine flow caused by fenoldopam alone, not when combined with enalapril, was higher on the HS (25.4±2.5) than the LS (13.5±2.8) diet (P<0.01, paired t test). Enalapril alone neither affected urine flow nor enhanced fenoldopam’s diuretic effect on either diet (Table 4A).

Urine Sodium Excretion (UNaV)
UNaV (baseline [vehicle, control] and enalapril) was greater on the HS than the LS diet and increased with fenoldopam39 on the HS (absolute and %, Table 4A, Figure 3A) but not the LS diet, as reported previously,22 despite less stringent salt restriction employed in the current study (50 mmol sodium/24 h) versus the previous study (10 mmol sodium/24 h).22 Enalapril alone did not affect UNaV on LS or HS diets (Table 4A, Figure 4A).40 The addition of fenoldopam to enalapril increased UNaV on LS but not HS diets (absolute and %, Table 4A, Figure 5A). Fenoldopam and enalapril caused a greater percentage increase in UNaV than fenoldopam alone on the LS diet (Supplemental Table 1, Figure 6A).

Control fractional excretion of sodium (FENa) was not significantly greater on the HS than the LS diet and increased with fenoldopam49 on the HS (absolute and %, Table 4A, Figure 3B) but not the LS diet, as reported previously,22 despite less stringent salt restriction employed in the current study (50 mmol sodium/24 h) versus the previous study (10 mmol sodium/24 h).22 Enalapril alone did not affect U_NaV on HS diets (Table 4A, Figure 4B). The addition of fenoldopam to enalapril increased U_NaV on LS but not HS diets (absolute and %, Table 4A, Figure 5A). Fenoldopam and enalapril caused a greater percentage increase in U_NaV than fenoldopam alone on the LS diet (Supplemental Table 1, Figure 6A).

Control fractional excretion of sodium (FENa) was not significantly greater on the HS than the LS diet (Table 4B) unlike U_NaV, related, perhaps, to creatinine clearance tending to be higher at baseline on HS than LS diets (percentage increase in FENa on a HS versus a LS diet =37.6±21.5). Fenoldopam increased FENa on HS but not LS diets (Table 4B, Figure 3B). Enalapril decreased FENa slightly on a LS diet but to a greater extent on a LS (34.2±9.5%) than a HS diet (3.5±17.3%; P=0.08 by paired t test; Figure 4B). The combination of enalapril and fenoldopam increased FENa on the LS but not the HS diet.
diet (absolute and %, Table 4B, Figure 5B) as with $U_{NaV}$. The percentage increase in $F_{Na}$ was greater with fenoldopam and enalapril than fenoldopam alone on the LS but not the HS diet (Supplemental Table 1, Figure 6, A and B).

Proximal Tubule Sodium Transport Assessed by Lithium Clearance
Baseline absolute and fractional lithium clearances (Table 4B) were similar on both diets, and were not higher on a HS diet, probably due to the acute water loading\(41\) (vide infra) employed to ensure adequate urine flow. On a LS diet, neither fenoldopam nor enalapril alone affected lithium clearance (Table 4B, Figures 3C, 4C). On a HS diet, enalapril did not affect lithium clearance (Table 4B, Figure 4C). By contrast, on a HS diet, fenoldopam increased absolute (Table 4B, Figure 3C) but not fractional lithium excretion because creatinine clearance with fenoldopam tended to be higher on HS than LS diets (Table 4A). On a LS diet, the addition of enalapril to fenoldopam restored fenoldopam’s inhibitory effect on sodium transport in the proximal tubule, increasing absolute and fractional lithium excretion (Table 4B, Figure 5C). On a HS diet, enalapril + fenoldopam did not significantly affect lithium clearance (Table 4B, Figure 4C). The percentage increase in lithium clearance with fenoldopam and enalapril was greater than with fenoldopam alone on LS but not HS diets (Supplemental Table 1, Figure 6A).

Absolute proximal reabsorption (APR) and absolute and fractional proximal sodium reabsorption (APR$_{Na}$ and FPR, %) were similar at baseline on both salt diets (Table 4, B and C) and were not decreased on a HS diet relative to a LS diet, which we attribute to the tendency of filtered sodium load to be higher on HS versus LS diets (21.4±18.1%).

### Table 2. Cardiovascular parameters on LS and HS diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>Control (C)</th>
<th>Fen</th>
<th>Post Fen</th>
<th>Enal</th>
<th>Fen + Enal</th>
<th>Post (Fen + Enal)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (BPM)</td>
<td>LS</td>
<td>62±2</td>
<td>62±4</td>
<td>62±2</td>
<td>63±3</td>
<td>60±2</td>
<td>62±2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>60±4</td>
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<td>t test, LS versus HS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>LS</td>
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<td>113±3</td>
<td>111±5</td>
<td>112±3</td>
<td>109±3</td>
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<td></td>
<td>HS</td>
<td>117±4</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>LS</td>
<td>66±1</td>
<td>59±3*</td>
<td>63±1</td>
<td>64±2</td>
<td>59±2*</td>
<td>64±1</td>
<td>*$P&lt;0.05$ versus LSC, LS Enal and Post Fen</td>
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<tr>
<td></td>
<td>HS</td>
<td>70±2</td>
<td>63±1*</td>
<td>71±1</td>
<td>70±2</td>
<td>61±2*</td>
<td>65±1</td>
<td>*$ΔP&lt;0.05$ versus HSC, HS Enal, Post Fen, RM ANOVA, Fisher’s LSD</td>
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<tr>
<td></td>
<td>t test, LS versus HS</td>
<td>NS</td>
<td>NS</td>
<td>*$P&lt;0.05$</td>
<td>*$P&lt;0.05$</td>
<td>NS</td>
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<td></td>
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<tr>
<td>Mean BP (mmHg)</td>
<td>LS</td>
<td>82±1</td>
<td>78±3</td>
<td>80±1</td>
<td>80±2</td>
<td>76±2*</td>
<td>92±7</td>
<td>*$P&lt;0.05$ versus LSC, LS Enal, Post Fen</td>
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<td>HS</td>
<td>88±2</td>
<td>80±2*</td>
<td>78±2</td>
<td>85±1</td>
<td>78±2*</td>
<td>84±3</td>
<td>*$ΔP&lt;0.05$ versus HSC, HS Enal, Post (Fen + Enal), RM ANOVA, Fisher’s LSD</td>
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<tr>
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<td>t test, LS versus HS</td>
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<td>NS</td>
<td>NS</td>
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</table>

Control (C), in response to fenoldopam (Fen), enalapril (Enal) and the combination of fenoldopam and enalapril (Fen + Enal), and postinfusion values [Post Fen and Post (Fen + Enal)]. Mean±SEM and statistical comparisons are as shown.

### Table 3. Renal hemodynamic parameters on LS and HS diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>Control (C)</th>
<th>Fen</th>
<th>Post Fen</th>
<th>Enal</th>
<th>Fen + Enal</th>
<th>Post (Fen + Enal)</th>
<th>Statistics</th>
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</thead>
<tbody>
<tr>
<td>PAH clearance (ml/min per 1.7 m²)</td>
<td>LS</td>
<td>624±25</td>
<td>702±74</td>
<td>527±22</td>
<td>726±110</td>
<td>714±47</td>
<td>568±66</td>
<td>$1^*$$ΔP&lt;0.001$ vs. HS C, HS Fen + Enal, RM ANOVA Fisher’s LSD</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>651±46</td>
<td>864±72$^*$</td>
<td>620±51</td>
<td>768±74</td>
<td>752±49</td>
<td>655±34</td>
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<td>t test, LS versus HS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Filtration fraction (%)</td>
<td>LS</td>
<td>15.7±2.2</td>
<td>14.9±2.3</td>
<td>18.1±3.8</td>
<td>18.0±2.5</td>
<td>16.6±2.3</td>
<td>18.5±3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>19.9±3.1</td>
<td>17.5±2.6</td>
<td>18.9±2.6</td>
<td>20.6±2.3</td>
<td>19.9±2.1</td>
<td>26.7±3.8</td>
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</table>

Control (C), in response to fenoldopam (Fen), enalapril (Enal) and the combination of fenoldopam and enalapril (Fen + Enal), and postinfusion values [Post Fen and Post (Fen + Enal)]. Mean±SEM and statistical comparisons are as shown.
### Table 4. Parameters of glomerular and tubular function on LS and HS diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>Control (C)</th>
<th>Fen</th>
<th>Post Fen</th>
<th>Enal</th>
<th>Fen + Enal</th>
<th>Post (Fen + Enal)</th>
<th>Statistics</th>
</tr>
</thead>
</table>
| Creatinine clearance (ml/min per 1.7 m²) | LS     | 104±14      | 101±16 | 96±12    | 120±13 | 115±13     | 115±13          | t test, LS versus HS NS NS NS NS NS NS NS **P=0.006 NS
<p>|                                  | HS     | 128±15      | 137±15 | 116±7    | 143±10 | 152±8      | 145±6           |            |
| Filtered sodium load (mmol/min)  | LS     | 15.4±2.0    | 14.8±2.2 | 13.9±2.1 | 17.5±1.8 | 16.8±1.8   | 16.8±2.4        |            |
|                                  | HS     | 17.1±2.4    | 1.2±2.6 | 16.4±1.5 | 20.6±2.3 | 20.2±1.0   | 19.7±0.9        |            |
| Urine flow (ml/min)              | LS     | 11.7±1.0    | 13.2±1.3*** | 10.6±0.8 | 11.8±1.1 | 14.7±1.9** | 7.9±0.5         |            |
|                                  | HS     | 12.3±1.1    | 15.5±1.7*** | 13.6±2.6 | 13.1±0.9 | 15.2±1.5*** | 12.1±1.6        |            |
| Lithium clearance (ml/min per 1.7 m²) | LS     | 26.1±2.8†   | 26.4±2.2 | 20.6±1.8* | 21.5±1.3 | 28.8±1.8*** | 23.6±2.5        | t test, LS versus HS NS NS NS NS NS NS NS *<em>P=0.01,<strong><em>P=0.001 versus LS, LS Enal, Post Fen, and Post Fen + Enal D‡P&lt;0.01,‡‡‡P&lt;0.001 versus HSC, HS Enal, Post Fen, and Post Fen + Enal RM ANOVA, Fisher’s LSD |
|                                  | HS     | 27.5±1.9    | 33.3±5.5</em> | 18.1±18.5 | 27.1±1.5 | 29.9±2.4   | 32.2±2.7        | t test, LS versus HS NS NS NS NS NS NS NS °P&lt;0.05 versus LS Enal, †P&lt;0.05 versus HSC, RM ANOVA, Fisher’s LSD |
| Fractional excretion of lithium (FELi, %) | LS     | 27.0±5.2†   | 31.9±8.1 | 31.9±7.9 | 20.0±1.9 | 28.2±6.7</strong></em> | 23.6±2.5        | t test, LS versus HS NS NS NS NS NS NS NS **P=0.01,<em><em><em>P=0.001 versus LS Enal, RM ANOVA, Fisher’s LSD |
|                                  | HS     | 23.1±3.4    | 30.0±9.6 | 24.4±6.1 | 21.1±2.7 | 21.3±1.7   | 22.6±1.5        | t test, LS versus HS NS NS NS NS NS NS NS °P&lt;0.05 versus HSC and HS Enal, RM ANOVA, Fisher’s LSD |
| Absolute proximal reabsorption, APR (ml/min) | LS     | 77.8±14.5   | 75.5±16.6 | 75.4±16.6 | 98.7±13.7</em> | 73.3±13.22 | 90.7±17.5      | RM ANOVA, Fisher’s LSD |
|                                  | HS     | 110.1±16.8  | 93.2±9.7</em> | 87.8±14.4 | 96.0±19.2 | 112.6±6.4  | 115.8±5.6      | t test, LS versus HS NS NS NS NS P=0.011</em> NS |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>Control (C)</th>
<th>Fen</th>
<th>Post Fen</th>
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<th>Fen + Enal</th>
<th>Post (Fen + Enal)</th>
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<tbody>
<tr>
<td>Absolute proximal reabsorption of sodium, APR$_{Na}$ (mmol/min)</td>
<td>LS</td>
<td>9.9 ± 2.1</td>
<td>10.2 ± 2.2</td>
<td>10.1 ± 2.6</td>
<td>12.5 ± 1.6*</td>
<td>8.9 ± 1.3</td>
<td>12.2 ± 2.3</td>
<td>*$p&lt;0.05$ versus Control, LS Fen, and LS Fen + Enal, RM ANOVA, Fisher's LSD</td>
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<tr>
<td></td>
<td>HS</td>
<td>13.8 ± 2.2</td>
<td>11.3 ± 2.2</td>
<td>12.1 ± 1.9</td>
<td>15.4 ± 1.5</td>
<td>15.9 ± 1.01</td>
<td>15.5 ± 0.9</td>
<td>$^3p&lt;0.05$ versus HSC, t test paired t test</td>
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<td>t test, LS versus HS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.036*</td>
<td>NS</td>
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<tr>
<td>Fractional proximal reabsorption, FPR (%)</td>
<td>LS</td>
<td>72.5 ± 5.2</td>
<td>70.1 ± 6.3</td>
<td>73.6 ± 3.4</td>
<td>78.1 ± 2.9</td>
<td>70.7 ± 4.7</td>
<td>76.3 ± 3.9</td>
<td>$^1p&lt;0.05$ versus LS Enal</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>77.3 ± 3.9</td>
<td>68.4 ± 5.6</td>
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<td>76.5 ± 2.8</td>
<td>76.6 ± 2.2</td>
<td>75.8 ± 2.0</td>
<td>$^3p&lt;0.05$ versus HSC, RM ANOVA, Fisher's LSD</td>
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<td>t test, LS versus HS</td>
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<tr>
<td>Distal sodium delivery (mmol/min)</td>
<td>LS</td>
<td>3.4 ± 0.3</td>
<td>3.3 ± 0.4</td>
<td>2.7 ± 2.4</td>
<td>3.3 ± 0.4</td>
<td>3.8 ± 0.2†</td>
<td>3.2 ± 0.2</td>
<td>$^1p&lt;0.05$ versus LS Enal,</td>
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<tr>
<td></td>
<td>HS</td>
<td>3.5 ± 0.2</td>
<td>5.1 ± 0.7</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>$^3p&lt;0.05$ versus HSC</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>(P = 0.053)</td>
<td>NS</td>
<td>P = 0.024*</td>
<td>P = 0.02*</td>
<td></td>
</tr>
<tr>
<td>Absolute distal reabsorption of sodium, ADR$_{Na}$ (mmol/min)</td>
<td>LS</td>
<td>3.7 ± 0.4</td>
<td>3.2 ± 0.3*</td>
<td>2.9 ± 0.2**</td>
<td>3.5 ± 0.3</td>
<td>3.7 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>$^*p&lt;0.05$ versus LSC, LS Fen, LS Fen + Enal</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>4.0 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>$\Delta p&lt;0.05$ versus HSC, Post HS Fen RM ANOVA, Fisher's LSD</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS (P = 0.06)</td>
<td>P = 0.03*</td>
<td>$^{\Delta}p&lt;0.01$ versus LS C and LS Enal</td>
</tr>
<tr>
<td>Fractional distal reabsorption, FrDR (%)</td>
<td>LS</td>
<td>94.7 ± 1.0</td>
<td>92.4 ± 1.2</td>
<td>94.2 ± 0.6</td>
<td>95.8 ± 0.7</td>
<td>93.8 ± 0.8</td>
<td>94.7 ± 0.7</td>
<td>$^4p&lt;0.01$ versus LS C and LS Enal</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>91.5 ± 1.1</td>
<td>87.4 ± 0.7</td>
<td>90.9 ± 1.4</td>
<td>92.4 ± 0.8</td>
<td>90.3 ± 1.7</td>
<td>92.3 ± 1.0</td>
<td>$^3p&lt;0.05$ versus HSC, paired t test</td>
</tr>
<tr>
<td>t test, LS versus HS</td>
<td>P = 0.04*</td>
<td>P = 0.004**</td>
<td>NS</td>
<td>P = 0.01**</td>
<td>NS</td>
<td>P = 0.002**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control (C), in response to fenoldopam (Fen), enalapril (Enal) and the combination of fenoldopam and enalapril (Fen + Enal), and postinfusion values (Post Fen and Post (Fen + Enal)). Mean ± SEM and statistical comparisons are as shown.
balance. Water loading could increase atrial natriuretic peptide, decreasing APRNa and FPR to a greater extent on LS than on HS diets; in the latter, atrial filling may not have such an impact as in the former.\textsuperscript{41} Fenoldopam alone decreased APR, APRNa, and FPR on HS but not LS diets (Table 4, B and C). Enalapril alone increased APR and APRNa on a LS diet (Table 4B) but did not affect FPR, as reported previously\textsuperscript{42} and related, perhaps, to the dose used and/or anti-natriuretic influences on the LS diet. Enalapril significantly increased fenoldopam’s inhibitory effect on APR, APRNa, and FPR on the LS diet and blunted fenoldopam’s effects on the HS diet (Table 4, B and C). Although the percentage decrease in APRNa with fenoldopam and enalapril was not greater than with fenoldopam alone on the LS diet (Supplemental Table 1), the median difference in APRNa between fenoldopam alone and fenoldopam and enalapril together was different from 0 in all subjects on the LS diet (\(P<0.03\), Rank-sum; Supplemental Table 2). As stated earlier, the increase in APR, APRNa, and FPR in response to enalapril on the LS diet obscured the effects of the combination of fenoldopam and enalapril when compared with the control values.

Baseline distal sodium delivery (Table 4C, Figure 7A) was similar on the LS and HS diets. Fenoldopam increased distal sodium delivery on the HS but not the LS diet, validating the notion that the inhibitory effect of fenoldopam on sodium transport in the proximal tubule is impaired during salt

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**Figure 3.** Fenoldopam (Fen) alone increases renal sodium excretion and lithium clearance only on HS. Data are shown as mean±SEM or median (range), with *\(P<0.05\) versus control (C) and postcontrol (not shown), RM ANOVA or Mann–Whitney/paired t test (LS versus HS), respectively. (A) Left to right, \(U_{NaV}\) (mEq/min) with control and Fen on LS and HS diets. Effect of Fen (\(\Delta\) Fen-C) on LS versus HS and percentage effect of Fen (% \(\Delta\) Fen-C) on LS and HS. (B) Left to right, fractional excretion of sodium, \(F_{ENa}\) % with Fen and control on LS and HS diets. Effect of Fen (\(\Delta\) Fen-C) on LS versus HS and percentage effect (% \(\Delta\) Fen-C) of Fen on LS and HS. (C) Left to right, lithium clearance (ml/min per 1.73 m\(^2\)) with Fen and control on LS and HS diets. Effect of Fen (\(\Delta\) Fen-C) on LS versus HS and percentage effect of Fen (% \(\Delta\) Fen-C) on lithium clearance on LS and HS.
depletion. Enalapril did not affect fenoldopam’s effect on distal sodium delivery on the HS diet, but increased it on the LS diet (Table 4C, Supplemental Figure 2). When combined with fenoldopam, enalapril also increased distal sodium delivery by a greater percentage compared with fenoldopam alone on the LS but not the HS diet (Figure 6A).

**Sodium Transport in the Distal Nephron**

Absolute distal reabsorption of sodium (ADR$_{Na}$) was similar on both diets at baseline (Control). ADR$_{Na}$ decreased during and after the fenoldopam infusion on the LS and HS diets (Table 4C, Figure 7, B and C).

Enalapril, both alone and in combination with fenoldopam, did not affect ADR$_{Na}$ on either diet, but blunted fenoldopam’s inhibition of distal sodium transport on both diets. Fractional distal reabsorption (FrDR) was lower on HS than on LS diets at baseline (Control). Fenoldopam alone decreased fractional distal reabsorption (FrDR) to similar extents on the LS and HS diets. On the LS diet, enalapril with fenoldopam tended to decrease FrDR ($P>$0.06 versus enalapril alone), but had no effect on the HS diet. The percentage decrease in ADR$_{Na}$ was greater with fenoldopam alone than with fenoldopam and enalapril on LS but not HS diets, although the directional changes were the same (Figure 8, Supplemental Table 1).
Potassium Excretion
Absolute and fractional (FE\textsubscript{K}) potassium clearances were higher on LS than HS diets (not shown). FE\textsubscript{K} was unchanged by fenoldopam, enalapril, or the combination of enalapril and fenoldopam on both diets, while postinfusion levels were decreased on the HS but not LS diet.

**DISCUSSION**

During moderate sodium load, renal dopamine exerts paracrine/autocrine inhibitory effects on proximal and distal tubular reabsorption of sodium.\textsuperscript{18–22,24–26,31,32,34,39} Selective gene deletion of aromatic amino acid decarboxylase (essential for renal dopamine synthesis) in the renal proximal tubule in mice produces salt-sensitive hypertension,\textsuperscript{43} underscoring the importance of dopamine synthesis by the proximal tubule in sodium homeostasis and BP regulation. Renal dopamine increases with acute and chronic salt loading\textsuperscript{44} and acts on D\textsubscript{1}-like\textsuperscript{45} and D\textsubscript{2}-like\textsuperscript{46} dopamine receptors, causing natriuresis.\textsuperscript{18–22,24–26,31,32,34,39} The natriuretic effect of dopamine is impaired in salt-depleted states.\textsuperscript{46,47}

We submit evidence, for the first time in normotensive (<120/80 mmHg)\textsuperscript{48} salt-resistant humans,\textsuperscript{33} that the RAS and dopaminergic system interact in regulating renal sodium transport on LS and HS diets. We confirm previous reports of natriuresis with D\textsubscript{1}-like receptor stimulation in normotensive humans on normal\textsuperscript{19} and HS\textsuperscript{22} diets, but not on LS diets.\textsuperscript{22,46,47}
In our cohort of salt-resistant normotensive humans, decreased mean BP in response to fenoldopam alone occurred on HS but not LS diets. When enalapril was added to fenoldopam, the mean BP decreased on both diets, suggesting that the RAAS could have mitigated the BP-lowering effect of fenoldopam during salt restriction.

Fenoldopam increases renal plasma flow on HS,50,51 and normal,52 but not LS diets,22 which was corroborated in the present study. Fenoldopam did not increase creatinine clearance on either diet, similar to previous studies that employed inulin clearance to measure GFR in normotensive humans.22,51,52 Fenoldopam does increase inulin clearance in hypertensive humans.53 Fenoldopam’s natriuretic effect on a HS diet is related, perhaps, to decreased proximal and distal sodium transport, as shown previously.22,24,32 Enalapril did not cause natriuresis on either diet at the dose employed. Higher doses (20 mg) of enalapril cause natriuresis in humans on 10–300 mmol Na+/day but not on 400 mmol Na+/day.54,55 A lower dose of enalapril (5 mg) in this study minimized any chance of an independent natriuretic effect that could confound the interpretation of the effects of enalapril combined with fenoldopam.

On a LS diet, fenoldopam did not independently cause natriuresis despite decreasing distal tubular sodium transport. This is attributable to the absence of an increase in distal sodium delivery, and the modest fenoldopam-mediated decrease in distal sodium transport. The increased effects of fenoldopam + enalapril on absolute and fractional lithium excretion on a LS diet compared with the effects of either treatment alone implicates the proximal tubule as the site of the interaction between the RAS and the renal dopaminergic system. Enalapril unmasked the natriuretic effect of fenoldopam and fenoldopam’s inhibitory effect on proximal sodium transport on LS diets, suggesting that heightened RAS activity in the proximal tubule during salt depletion in humans, as in animals,56 inhibited fenoldopam’s natriuretic effect. Lithium clearance may underestimate proximal tubular sodium transport when employed as its surrogate indicator during salt- and volume-depleted states because of lithium reabsorption beyond the proximal tubule. Increased renal nerve activity during salt depletion could also oppose fenoldopam’s effects on renal proximal tubular sodium transport, which warrants further investigation. During salt repletion, continued reabsorption of lithium beyond the proximal tubule does not generally occur. Therefore, the increase in lithium clearance with fenoldopam alone on a HS diet indicates inhibition of proximal tubular transport by fenoldopam during salt loading, with the caveat that lithium clearance has significant intra-individual variability, irrespective of salt intake.60

Figure 6. The combination of fenoldopam + enalapril (Fen+Enal) has a greater effect in decreasing sodium transport in proximal nephron than fenoldopam (Fen) alone on low salt. (A) Fen + Enal causes a greater percentage increase in UNaV, FENa, lithium clearance and distal sodium delivery than Fen alone on low salt. Percentage changes in UNaV, FENa, lithium clearance and distal sodium delivery on LS and HS are shown, *P<0.05, one-way ANOVA, Bonferroni. Data are expressed as percentage effects compared with pre-infusion values, mean±SEM. (B) Timed graph showing that the increase in FENa is greater with Fen + Enal than Fen alone on low salt. Timed graph of the increase in the ratio of FENa before (control) and during fenoldopam infusion on LS with or without pretreatment with Enal. *P<0.05, versus control and LS Fen two-way ANOVA, Bonferroni post-hoc.
Normotensive humans on HS diets exhibit decreased sodium transport in proximal and distal tubules when treated with fenoldopam. In the present study, salt-resistant normotensive humans on a HS diet also had decreased absolute and fractional proximal and distal sodium transport in response to fenoldopam (a nonselective D1 and D5 receptor agonist). The absence of kaliuresis in the face of increased distal sodium delivery in response to fenoldopam on both LS and HS diets suggests that D1-like receptor (likely the D5 receptor) activation occurred at more distal sites in the nephron. Enalapril had no effect on distal sodium transport and decreased the effects of fenoldopam on distal sodium transport on both diets, the mechanism of which needs elucidation.

The addition of enalapril diminished fenoldopam's natriuretic effect on HS diets by blunting its effects on proximal tubular transport, the reasons for which are unclear. We speculate this may be related to a decrease in the number of D1-like receptors62,63 in the proximal tubule available for receptor-receptor interactions between the D1-like receptor and the AT1 receptor after 5 days on a HS diet, which we will study in the future.

Our rigorous mechanistic study is limited by the small number of subjects, so our results must be interpreted...
cautiously. Some statistical tests were underpowered to detect a difference between the groups compared. The use of creatinine clearance as a measure of GFR instead of inulin (which is no longer approved for human use in the United States) is a limitation. Subjects refused a urinary catheter. Half-hourly urine clearances do not always represent a fully evacuated bladder, which is a limitation. Lithium clearance may not be predictive of end proximal tubular sodium outflow due to some reabsorption in the loop of Henle. The use of exogenous lithium and a water load may underestimate subtle differences in lithium transport, which was addressed by the crossover design of the trial.

In conclusion, in salt-resistant normotensive humans on HS diets, fenoldopam causes natriuresis by inhibiting proximal and distal tubular sodium transport, similar to the effects of low doses of dopamine in humans. The absence of natriuresis in response to fenoldopam during modest salt restriction compared to its robust natriuretic effect during salt loading may be attributed, in part, to increased RAS activity suggested by the increased percentage effect in response to fenoldopam and enalapril, compared with fenoldopam alone, on a LS diet. This is the first translational confirmation in salt-resistant normotensive humans of a negative interaction between the renal dopaminergic system (via D₁-like receptors) and the RAS (via the AT₁ receptors) in the proximal tubule that may influence sodium transport on LS diets, hitherto only described in in vitro and in vivo animal studies. These findings support the notion that dietary salt restriction stimulates the RAS, providing a mechanism by which dietary salt restriction fails to enhance the natriuretic effect of agents acting beyond the loop of Henle.

CONCISE METHODS

Recruitment
The protocol and consent forms (Supplemental Material 3) were approved by the Georgetown University Medical Center Institutional Review Board. The trial was conducted between November 2003 and June 2006. Volunteers who responded to advertisements in the community were screened by a telephone questionnaire. Written informed consent was obtained from all eligible volunteers (Supplemental Material 3), followed by a history, physical examination, and screening investigations, including lipid profile, urine protein/creatinine ratio, chest x-ray, and electrocardiogram conducted in the Clinical Research Unit (CRU) of the Georgetown University Medical Center. Eight normotensive subjects between 18 and 55 years of age with body mass index within 20% of ideal body mass index for age, who met screening criteria (Supplemental Material 1) and BP<120/80 mmHg were enrolled. The mean of three recordings of BP, two using the right arm and one using the left arm, measured by an attending physician, using a mercury sphygmomanometer (TRIMLINE, Branchburg, NJ), in the seated position (at least 5 minutes) in a quiet room, was used to determine BP. Participants were each assigned a random subject number. The pharmacist and dietician were privy to the nature of each intervention while the subjects, nurses, and physicians in the CRU were blinded to the intervention.

Diet
The subjects were placed on an isocaloric diet with 1 g protein/kg body weight, containing low (50 mmol sodium/24 h) and high (300 mmol sodium/24 h) NaCl for 5 days each, with a washout period of 4 weeks: each subject acted as his/her own control. Subjects reported daily to the CRU for diet collection, monitoring of weight and BP readings, and submission of 24-hour urine for volume, sodium, potassium, and creatinine. When sodium balance was achieved (24-hour urine sodium = daily intake), typically on Day 5, but up to Day 7 of the diet when necessary, the subjects were admitted to the CRU and treated with a low dose of enalapril (2.5 mg) or placebo every 12 hours in a counterbalanced order. Subjects spent the night in the CRU, continuing on the diet until 10 PM on Day 5 with heart rate and BP monitored by a registered nurse, using Dinamap Procare 200, validated by the European Society of Hypertension.

On Day 6, the subjects remained fasting except for a 20 ml/kg water load, a tracer dose of lithium (lithium carbonate, 600 mg) and enteral sodium and potassium supplements to match their current dietary sodium and potassium intakes and water intake to replace urinary losses every half hour. When subjects attained water balance (two consecutive voids of urine less than 100 ml difference from each other in volume), an intravenous infusion of PAH was begun to measure effective renal plasma flow. Cardiovascular parameters, urine flow, sodium, and potassium, and creatinine, PAH, and lithium clearances were measured every half hour with the subjects in the supine position, using standard analytical methods, before (control), during (experimental), and after (postcontrol) a 3-hour fenoldopam infusion administered at 0.05 μg/kg/minute. On Day 7 the trial was repeated with the opposite intervention, i.e., enalapril/placebo.
Data Analyses and Statistics
All data are expressed as mean±SEM except when otherwise specified. Hemodynamic parameters including heart rate and systolic and diastolic BP were measured before, during, and after the fenoldopam infusion. Creatinine, lithium, PAH, potassium, and sodium clearances (ml/min per 1.73 m²), fractional excretions of sodium (FE_{Na}), lithium (FE_{Li}), and potassium (FE_{K}), fractional (FPR) and absolute proximal reabsorption of sodium (APR_{Na})), distal sodium delivery, and fractional (FrDR) and absolute distal reabsorption of sodium (ADR_{Na}) were measured\textsuperscript{22} calculated\textsuperscript{24} as reported previously. Baseline values represented the mean of the pre-infusion values. The total of all values during the prefenoldopam (control), the fenoldopam infusion and the post-infusion period were analyzed using two-group comparison tests or Wilcoxon Rank-sum test when applicable. Details of the clinical protocol are in the online supplement.

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DISCLOSURES

None.

REFERENCES


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